PHYSIOLOGICAL REVIEWS

VOL. XIII OCTOBER, 1933 No. 4

PHYSICAL FACTORS IN BODY TEMPERATURE MAINTENANCE AND HEAT ELIMINATION

THOMAS DEIGHTON

Institute of Animal Nutrition, University of Cambridge

The last few years have been noteworthy for a change in the mental position of physiologists in this matter, moving always further from vitalistic ideas toward the admission of physical and usually simple explanations. The culmination of this tendency in the work of Bohnenkamp (1) and his collaborators, whose genius makes heat elimination directly deducible from considerations of Lambert's Equation, the Universal Radiation Constant and the Stefan-Boltzmann relation is a milestone on the road of progress in metabolic research, the brilliancy of which no criticism of details can dim.

In the ensuing pages no attempt has been made to provide a historical basis or general bibliography. These general desiderata of a review are supplied by Barbour (2) for heat regulation generally, at a later date by Gessler (3) and preëminently last year by Stigler (4) with nearly four hundred references. The literature cited herein has, in the main, reference to recent work of importance in connection with the restricted subject matter treated and the arguments developed are kept as free as possible from mathematical equations. Reference to any other results mentioned will be found in one or other of the three articles above named.

In this essay only a small part of the subject of heat regulation is considered, namely, the effect of physical factors on heat *elimination*, in animals which are provided in some considerable degree with a mechanism for maintaining the body at a constant temperature. Gessler (l. c., p. 209) prefers to consider this as a mechanism for maintaining a constant heat content (Wärmebestand) in the body, but were this the case one would anticipate far greater temperature variations than actually occur, corresponding to known weight variations. Moreover

special conditions would have to be postulated for children, changing as they grow to manhood. Furthermore the possible mechanism of such a "caloristat" is very difficult to imagine. Perhaps one is intended to assume the heat as reckoned per unit weight; this would rob the first objections of much of their point but the last remains. In any case it would be very difficult to demonstrate any superiority for this conception over constancy of temperature since a variation of half a degree in mean body temperature would be caused, other things remaining constant, by a variation in heat content of $\frac{\frac{1}{2}}{273 + 37} = \frac{1}{620}$.

Heat regulation is effected by variations in both heat production and heat elimination and the way in which these two react to changes in the physical, chemical and physiological conditions provides a wide field of research which has been diligently cultivated in the last year or two. Heat production, on which its elimination must ultimately depend, is doubtless affected by variation of any physical factor whatever and evidence supporting this statement is forthcoming in the experiments of Otani (5) on magnetic fields and high pressures and those of Schliephake (6) on dielectric strain.

When the present knowledge of the constitution of atoms and molecules is considered this is by no means to be wondered at, but it remains true that few of these physical factors have a large effect and of these the most important are the law of mass action and van't Hoff's law, since any surface effect or nervous control must without question remain subject to the former, while the operation of the latter is very distinctly traceable wherever body temperature *variations* ensue. Recently Bělehrádek (7) has questioned the general applicability of van't Hoff's law in biological phenomena considering that the phenomena are better explained in terms of viscosity variations in protoplasm. His theory accounts notably for variation of the temperature coefficient of metabolism with age, while changes which have been shown to take place in protoplasmic viscosity during mitosis are mirrored in the changes in temperature coefficient of mitotic change.

Elimination of heat on the other hand is largely controlled by temperature and humidity differences in the air and in the superficial portions of the animal, the latter being largely under vasomotor control. Of high importance is the extent to which an animal is clothed or covered with fur, hair or other substance of small thermal conductivity. It would appear also to be a necessary conclusion from the high percentage (80 per cent) of heat loss found by Rubner (8) to be due to radiation

from an animal, a figure later confirmed by Bohnenkamp and Ernst (1) that the amount and character of infra-red radiation received must have a notable effect on heat elimination by this means since a good radiator is always a good absorber. Rubner (9) in a recent publication envisages the possible effect of sunlight in this regard by enumerating insolation among the factors influencing thermal environment from the physiological viewpoint. See also remarks p. 455 on the effects of wall temperature of rooms.

The sharp gap between warm- and cold-blooded animals is bridged by a few intermediate types investigated in the well known work of Martin (10). Kolmer (11) denies the truth of Martin's statement that monotremes are devoid of sweat glands and thinks that physical regulation takes place by evaporation of formed sweat as yet unsecreted. A better explanation may probably be found in some colloid change in the skin analogous to that found in man by Schade and Marchionini (12) which is independent of the existence of sweat glands, combined with an ability to alter the volume of the insensible perspiration observed in man by Borra (13) and later in a measure confirmed by Kayser, (14) an ability which is also independent of sweat secretion.

Hall and Root (15) have recently published some interesting experiments on amphibians and reptiles which are of interest as indicating the possibility that the body temperature of warm-blooded animals may be affected by humidity changes in the surrounding air; especially under such conditions as are accompanied by or tend to induce an approach to poikilothermy as in the newly born and in narcosis. It appears from the experiments of these investigators that in very dry air of relative humidity about 7 per cent the body temperature of amphibians may fall 7° to 9°C. *below* that of their surroundings, while that of reptiles is affected to the extent of less than one degree. It is suggested that the development of a horny skin may have been a step in the evolution of homoiotherms. Benedict and Fox (16) have shown that the giving off of water vapour is the principal means of heat elimination in the python and Kayser's view (14) that the abnormally high water: oxygen ratios of some small animals such as doves and rats when young is due to water arising from increased metabolism is in agreement with the forementioned idea, more especially as the values obtained approach those of poikilotherms. Adolph (16a) continuing the work of Hall and Root has shown that in the frog this effect is independent of nervous control, circulatory disturbances, etc.

Barbour (l.c.) and Du Bois (17) both emphasize that in fevers the

metabolism rises with body temperature in accordance with van't Hoff's law and that there are certain infections and neurogenic fevers in which a vicious circle is set up, the disease itself causing a partial failure of heat elimination. Whether increase of body temperature always acts on the metabolism in accordance with Van't Hoff's law is however not certain. Radsma (18) has shown that the metabolism of Europeans in the tropics is often as much as 10½ per cent below the Benedict standards although the body temperature is higher. These results are confirmed by Radsma and Streef (19) who further show that this reduction is not due to lower specific dynamic energy input as the result of lower protein diet or to differences in carbohydrate or fat proportions such as were considered by Krogh and Lindhard. The work of Bělehrádek has already been referred to. These and other results[1] make it questionable how far it may be legitimate to use body temperature as a scientific datum. Maresh (20) finds half a degree topographic difference in the temperature of the visceral organs of rabbits, while the high liver temperature and peripheral temperature gradients are well established. Nevertheless there remains little doubt that there is a temperature which may be defined within pretty narrow limits in man and rather wider ones in other cases which is characteristic of each warm-blooded animal. This temperature is subject to *small* variations due to the interplay of physical, chemical and physiological factors and especially is this noticeable when one of these factors is varied over a wide range as in Hashimoto's (21) experiments in cold packs. This suggests that the body temperature might be higher in the tropics as found by Radsma (l.c.). The further deduction that owing to their skin's greater absorptive power the temperature of pigmented peoples should be higher, at any rate in sunshine, than that of whites under like conditions seems to be negatived by the results of many observers quoted by Stigler (22). This is possibly linked with the surprisingly high coefficient of absorption of white skin for long wave heat rays observed by Abbot and others (see p. 454) and with sweating.

The observations of Stigler (l.c.) raise the further question how far one is justified in grouping warm-blooded animals in a single class, since he has succeeded in raising the "thermostat setting" in rodents and keeping them for a long period at the higher level. The same experiment on other warm blooded types led to rapid death from heat

[1] Those of Matakas (Arch. Exp. Path. u. Pharm., clxii, 395 (1931)) appear to have a notable bearing here adequate discussion of which is prevented by considerations of space.

stroke, by the operation of van't Hoff's law. Gudjonsson (23) has caused a permanent *lowering* of the thermostat setting by avitaminosis in rats. Further evidence that some further subdivision of warm-blooded animals in this matter is needed is afforded in the work of Hesse (24) on the means of temperature regulation in the African elephant which appears to be provided with a special heat eliminator in its large vascular ears, regulation being effected by varying the rate at which they are moved back and forth. Though it cannot be artificially induced something of the nature of the raising of the thermostat setting in man is exemplified in the course of affairs after the rise of temperature in typhoid fever (see also p. 439).

Benedict and Shell (25) note that fasting in men does not greatly affect body temperature and the observation has been confirmed many times since then. In fasting experiments with pigs (unpublished) in a calorimeter I have consistently found the opposite to be the case and invariably note temperature falls usually of two or three degrees F., sometimes more in the course of a five day fast. Benedict and Shell noted a lowering of about a degree when a fast was preceded by hard work but the pigs mentioned are always kept in pens for a long period before experimentation. Benedict and Ritzman's (26) experience with undernourished steers agrees with that of Benedict and Shell but some lowering of skin temperature was found and similar results were observed in fasting animals (27) and in Benedict's historical observations on Levanzin (28).

The daily rhythm, the limits of which are set by Gessler as 36°.5 to 37°.5 C. in man, the former being observed in the early morning and the latter in the early afternoon is pretty well established. Two groups of individuals differ from this generalization, namely, new-born babies, which are sub-poikilothermic and the very aged whose mean according to Chelmonski is rather lower and in whom the daily rhythm is often reversed. Johansson (29) in his classic paper endeavours to show that this rhythm is entirely attributable to two causes, viz.—muscular activity and food intake each of which would he thinks tend to place the minimum where it is in fact found. Gessler is unable to believe that the odd half degree variation still found by Johansson, even in the quietest experiments, is due to involuntary movement and points out that were this so a reversal of the usual phenomena should be shown by night workers on which point the results of Lindhard (30) and those of Benedict and Shell (28) are at variance. The latter investigators found that after hard work there was a definite lowering of body temperature and

Gessler considers this fact to be at the bottom of the apparently contradictory findings as Lindhard used hard manual workers. Gessler thinks that the above lowering after work masked the normal daily rhythm which he opines remained unchanged as in the experiments of Benedict and Shell.

The question seems to be still undecided. An important point which I believe has received less attention than it should have is the *actual time* of minimum. All investigators agree in putting this at 2–4 a.m. Now this would be comprehensible on Johansson's theory if it could be established—(a) that the metabolism was at a minimum at about the appropriate time after food, and (b) that sleep was most deep between these hours. In point of fact the evidence is against both these propositions. All investigators agree on the practically continuous fall of metabolism in the period from the fifth to the fifteenth hour after food, and sleep in man is deepest according to Kohlschütter, since confirmed by many others (31), about two hours after its commencement. The hours 2–4 a.m. are noticeable for a very light slumber which becomes deeper again later on. The minimum of Kohlschütter is excellently shown in many of the curves recently given by Endres and Frey (32) especially in cases where there has been considerable muscular activity the previous day. These facts do not support Johansson's theory.

One fails on the other hand to follow Hári (33) in thinking that since the body can at all times guard against violent temperature fluctuations, the central controlling mechanism is evidently at work and therefore that it must "acquiesce" in the temperature fluctuation. It seems to the writer more reasonable to adopt one of several hypotheses which do not demand such a dual function on the part of the regulating centre or alternatively a dual centre. It may be for example that the true normal "thermostat setting" of the central regulation is actually 36.5°C. the slight excess over this at various times being due to prolonged slight after effects of other factors which only pass off in deep sleep. The restlessness between 2–4 a.m. may on this assumption be precisely the cause of the subsequent rise before waking, and Johansson's theory would then fit the facts quite well, more especially if there is any truth in the modern psychological theory of "action patterns" as an explanation of thought (34) as it is then unnecessary to postulate definite movement, dreaming would suffice. Fantastic as this seems at first sight it accords remarkably well with the results of all experiments so far made on the effect of mental work on metabolism. The earliest results of Benedict and Carpenter (35) showed a slight increase of metabolism during mental work whose significance was questionable.

Becker and Olsen (36) in their well known paper on the same subject found a considerably higher CO_2 elimination. Later work by Benedict and Benedict (37) confirms the earlier results of Benedict and Carpenter leaving a very small amount, two or three per cent only, to be divided between observed heart and respiration rate increase and mental work effect, if any. Such small increase is just what might be expected from the full contraction under the "all or nothing" principle of a few isolated muscle fibres as postulated in the theory of action patterns. Rosenblum (37a) obtains results sensibly in accord with those of Benedict and Benedict.

Gibson (38) and Osborne (39) established the fact that the rhythmic change in body temperature always follows local time as far as experiments in travel allow this to be tested.

The most recent experiments on the day and night periodicity in body temperature were carried out with much care by Völker (40). He found that in normal fasting men the minimum temperature is recorded between 2–4 a.m. while the maximum persists on the average from 1 p.m. to 7 p.m. practically unchanged while the 11 p.m. observation was equally high. In men on a normal diet the *rise* of temperature in the early morning never occurred in sleep and the rise from the time of waking was continuous right up to late evening when the subject again retired to rest. By inverting the conditions he was never able to secure a true reversal of rhythm, at most the minimum was somewhat displaced. Völker asserts that the same periodicity with the same local time relation, which is quite unaffected by the time of year, height of sun or duration of daylight (he experimented in Hamburg and at Akureyri in Iceland) is observable in the pulse, the blood pressure, the metabolism (I have long suspected this in pigs), the kidney secretion, the nyctinastic movements of the cotyledons of Phaseolus, and the electrical conductivity of the atmosphere. Subsequent experiments by Bunning, Stern and Stoppel (41) show that this last is *definitely not the cause* of the periodicity in plants, and certainly it cannot have been in the case of the pigs under experimentation here as they are screened by being enclosed in a metal walled calorimeter. This is quite in consonance with Völker's expectation; he pointed to some unknown cosmic cause for all such diurnal variations.

An interesting letter in a recent number of "Nature" (42) brings to light a singular observation on the North Greenland expedition which appears to have an immediate bearing on the present matter. Cook observed that Eskimo women were subject to an annual menopause in

winter and that the men were temporarily impotent and lacked all sexual desire, experiencing also great muscular weakness. Marshall (43) had earlier drawn attention to similar phenomena in many animals and the matter has been pursued by Ramanathan (44). That this was not a racial factor was clear as the members of the expedition were equally affected. These phenomena are such as accompany hypopituitarism, a normal concomitant of which is also a lowered basal metabolism and body temperature. The stimulus in spring is in this case assumed to be *light* and it would be most interesting to see Völker's experiment repeated in Spitzbergen or Northern Greenland as the Akureyri experiments seems to me susceptible of criticism on one point.

Although full daylight would be experienced at midnight in June and July the sun was not actually above the horizon at local midnight even on 27th June, 1925, the nearest experimental date to the solstice. Reckoning to the nearest minute of arc we have for this day—declination 23° 20′, refraction 35′, sun's semidiameter 16′, and dip of the horizon at a man's height 2′. The sun's equatorial horizontal parallax is negligible. Hence the most southerly latitude in which the midnight sun was visible on this day was 65° 47′ N. at sea level. Even if Völker's laboratory were at 50 feet elevation, sufficient, that is, to bring the upper limb of the sun above the horizon, Dorno (45) has shown that the amount of solar radiation transmitted is negligible up to an altitude of several degrees. A more northerly station would moreover be better on account of the lesser difference between midday and midnight altitudes of the sun and if winter as well as summer and midseason observations could be taken the value of the work would be greatly increased. Winter observations in Hamburg showed a minimum at 4 a.m. while moonlight on the North Greenland expedition was without effect.

There is another electrical phenomenon which has recently been shown to be related to the basal metabolism and so one may anticipate, in spite of Radsma's work, generally correlated with the body temperature, namely, the electrical conductance between different portions of skin. Purdy, Johnson and Sheard (46) find that the basal metabolism is equal to

$$\frac{\log y - \log 0.005}{-\ 0.0396}$$

when y is the electrical potential difference between the area of skin over the articulation of the ulna and radius at the wrist and that at a

point situated 12 cm. higher up the arm. Thus the higher the basal metabolism the lower the potential difference and the higher the *skin* conductance; whereas in Völker's work the higher the *air* conductance the lower was the body temperature and presumably the metabolism. Purdy, Johnson and Sheard have connected this effect with the blood flow which would appear to be the common link between the two since a reduced flow whether due to artificial restriction by arterial compression or to hypothyroidism affects the potential difference in the same sense to a marked degree.

Gessler (47) attempts to explain the daily rhythm on the supposition that the stimulability of the mechanism is greater by day, since the response to pulling off a number of blankets is greater by day than by night when the room temperatures are about the same and other conditions similar.

One may well keep in mind however that the "cosmic cause" of Völker may operate either upon the central regulating mechanism or the afferent or efferent nerves themselves since any factor capable of affecting the ionization of the air may not inconceivably have some effect on nerve operation.

Bissonette (48) has recently traced the periodic variations in age at sexual maturity in birds to time, intensity and wave length factors of light, variations being ascribed to changes in the relative amounts of long, stimulating and short, inhibiting waves.

Gessler relies on a conception of greater winter stimulability to explain a supposed seasonal rhythm. He thinks also that the day and night reversal in the aged may be due to the fact that the hours of sleep are usually much curtailed in these cases. It remains true however that in most cases, even then, more sleep is taken by night than by day.

Gessler notes that the diminished stimulability in summer does not cause a diminished body temperature but that we do not know definitely whether the *mean* temperature of the body is not lower. On the other hand neither do we *know definitely* whether there is any seasonal variation to explain. Gessler's work (49) has been criticised by Völker on the grounds that insufficient attention was paid to control of conditions. Lately however Wachholder and Nothmann (50) think they have established an annual periodic change in function of certain muscles which may turn out to be of importance from this standpoint. Völker (l.c.) was unable to find any seasonal effect. Gessler's curves correspond with such exactitude however that it is difficult to believe that there was not actually some effect under the conditions under which he worked—

this was by short experiments while the subject was living a normal life. Any possible "carry-over" effect of the cold weather outside in winter would certainly make itself felt under these conditions. Here in Cambridge I have never yet established any seasonal effect on the metabolism of castrated pigs kept in the dark for some days. Recent work by Ogle and Mills (50a) on heat adaptation and by J. Giaja (50b) on fatigue renders the existence of such a carry-over effect by no means unlikely. Maignon and Guilhon (51) found a distinct seasonal variation in the basal metabolism of dogs kept at constant temperature. The maximum was in May.

Benedict and Ritzman (52) working with steers found a distinct seasonal effect, even in their control animals, apparently depending on temperature *but in the opposite sense to that of Gessler.* The main question is thus anything but settled.

We may then consider that apart from very minor variations the temperature of the human body in health keeps constant within about a degree Fahrenheit. From very extensive experience of the rectal temperatures of pigs in this laboratory it appears that temperatures from 101°F. to 103.5°F. must be looked upon as normal. Thus the range of variation is greater than in human beings. Tereg (53) gives among others a range of 2°F. for the ass, 3.5° for cattle and dogs and even 5° for poultry. Hence man may represent the limit to which an evolutionary trend to homoiothermy has attained, ordinary domestic animals carry us a stage further back and the instances mentioned p. 429 represent a more remote adjustment. Marshall (54) puts forward the alternative suggestion that the high variability of body temperature may be connected with the almost complete absence of sweat glands in those animals which exhibit it. He thinks that unwonted exercise may well raise the body temperature over a longer time in the case of animals like the cow which have no means available for cooling the body rapidly as the horse and, in a different way, the dog have. He points to the great discomfort suffered by cattle when compelled to exert themselves violently for some time. That man and dog have heat regulatory mechanisms suited to rather different climatic conditions has been rendered clear by Dill (54a) and his co-workers. The former conception lends interest to the experiments of Pembrey (55) who showed many years ago that a mouse showed a rapid regulatory reaction against cold. On re-heating, the opposite reaction was much slower (cf. remarks on possible carry-over effect, above.) Very young blind mice on the other hand acted as poikilotherms and followed van't Hoff's law as to heat

production. At eight days old a neutral point was reached. Chicks reached this stage before hatching, while young pigeons which are not active when first hatched went through the stage mentioned subsequent to hatching. Recently Ginglinger and Kayser (56) claim to have established three classes of animals. The first, of which the mouse and dove are examples, comprises those born naked without fur or feathers, without thermo-regulation and resembling poikilotherms, in which heat regulation develops later, the chemical before the physical. The second class, of which examples are the guinea pig and hen, are born or hatched fully able to fend for themselves and already have both types of heat regulation. The third type is exemplified by rabbits and cats which are born more or less helpless, are semi-naked and have chemical but not physical regulation developed. The fact of the earlier appearance of chemical regulation in the life history has been further rendered clear by the experiments of Kayser (14) wherein he shows that the water:oxygen ratio is equal at the critical temperature for all the animals used, namely, the tortoise, mouse, rat, rabbit, guinea pig and dove and that for any one species it is constant at any one temperature. In growing rabbits at 20°C. fall in temperature (30°–10°) is mainly covered by the physical regulation while in the dove the "water metabolism" remained unchanged, the total metabolism rising 93 per cent. Even in young rabbits the $\frac{H_2O}{O_2}$ ratio was diminished. In doves and rats on the other hand this ratio was often twice the adult value and approached values attained for poikilotherms. These results further confirm the deductions of Martin (10) on the order of development of the chemical and physical regulations.

We may however venture to doubt how far the classification will be able to be maintained in the future;—the rodent class of Stigler (22) cuts clean across this division, but refers of course to exogenous hyperthermia which may not follow the same laws. We must moreover guard against pushing the analogy between the first class of Ginglinger and Kayser and the cold-blooded animals too far. Gessler has already drawn attention to the danger, in that cold-bloodedness is not merely absence of the normal heat regulatory mechanism of homoiotherms since many cold-blooded creatures find it impossible to live at 37°C. and one might with almost as much justification point to the even better established fact that very young homoiotherms have to be very carefully protected from the natural consequences of their condition or they die even more easily than poikilotherms at 37°C.

That the difference between the two goes deeper than the mere presence or absence of a regulatory mechanism is shown by the recent work of Nicloux (57) who has demonstrated that the rate of alcohol absorption is very different in the normal mouse and in cold-blooded animals. In the latter the rate of absorption varies according to van't Hoff's law with a temperature coefficient = 2, while in the latter it is constant and about ten times as great under normal conditions reckoning the mean temperature of the mouse as 30° (say).

The thermostatic mechanism is undoubtedly assisted materially in performing its functions by the large water content of the animal body. As pointed out by Barbour its high specific heat, latent heat of evaporation and thermal conductivity render it peculiarly suitable for the "damping out" of violent fluctuations in temperature. It seems worth while however to try to get a more precise idea of the physical points here involved. Suppose one part of an organism to be overheated as a whole; its temperature may be reduced by evaporation of water from its surface, by the flowing away of warm blood from its capillaries and its replacement with cooler and by a corresponding flowing away and replacement of the lymph which laves the cells of the tissues themselves, together with small amounts carried from the surface by conduction, convection and radiation. If however this alone took place we should soon have a cool surface, cool capillaries and the cell surfaces cooled to a greater or less extent according to the amount of lymph passing over them. The insides of the cells and of masses of adipose or other less vascular tissue would remain warm were it not for conduction through the tissues to the surface and to the blood and lymph stream. Now in any static system the *thermal conductivity* alone determines the heat flow, but in a constantly varying one the correspondence is lost, and the *diffusivity*, which is a function of thermal conductivity, specific heat and density equal to

$$\frac{\text{conductivity}}{\text{spec. ht.} \times \text{density}}$$

comes more and more to the fore as the determining factor. It is this which controls the rate at which a steady temperature gradient is established and so it is of primary import in the case with which we are dealing. It is clear that the high specific heat tends to reduce diffusivity but this is more than counterbalanced by the high thermal conductivity of water by comparison with organic liquids. Hence on the whole the diffusivity is also considerably higher than that of other

liquids, so that from this standpoint also water is the most satisfactory fluid constituent for the body of an homoiotherm.

The fact that it is possible to raise the heat production of an animal but not to lower it below the level of fasting katabolism seems to indicate that the regulating mechanism must operate on the eliminating arrangements and J. Giaja (58) has gone so far as to claim that the basal metabolism represents the minimum, not for continuance of life but for maintenance of homoiothermy. In practice however the two may well amount to the same thing. That the eliminating mechanism is disturbed in fever is clear. Barbour quotes Söderström and Du Bois' experiments in which a typhoid patient in the rising stage showed a reduced H_2O output, while in the following stage this was increased.

The body temperature may be increased, temporarily, by the performance of muscular work and by the specific dynamic action of food. Gessler considers that such changes must be considered not to interfere with heat regulation, by which he presumably means that they do not throw the mechanism out of order. That it takes full cognizance of and allows for them in a variety of ways is certain. Increased perspiration, flushed skin, deeper and more rapid breathing testify to this in the case of muscular work. As regards food Anderson and Lusk (59) found some fifteen years ago that there was a certain reciprocity in regard to carbohydrate utilization as between heat production and the performance of mechanical work in the dog. Benedict and Mürschhauser (60) found no such effect in man. The writer (61) has found an effect in pigs similar to that of Anderson and Lusk and of greater magnitude, heat elimination in the early days of fasting and muscular work done being negatively correlated.

The actual measurement of body temperature is not easy. The difficulties are reviewed by Stigler (62) who recommends the statement in the case of rectal temperatures (a) of the depth of insertion, and (b) of the outer temperature, owing to the considerable effect of temperature gradient. Clearly as we go down the scale as far as, say, the mouse as in Nicloux's work, it becomes very problematical what poikilothermic temperature it should be compared with owing to the complication introduced by the existence of this gradient.

In spite of the complications of diffusivity it is clear that there must be something in the nature of a *mean temperature gradient* in the tissues of homoiotherms. This has been investigated by Bazett and McGlone (63) with considerable thoroughness by means of thermocouples inserted below the skin of the forearm and thigh. This work brings out

clearly first, that the gradient varies with the subject, secondly that there is a change at the position of the deep fascia, and thirdly that the sensation of "feeling cold" is associated with *cooling of some layer* even when the surface is increasing in temperature as after a cold bath. This would appear to afford an explanation of the stage of "chill" in fevers as due to cooling of the tissues immediately below the skin due to adjustment of temperature gradient on withdrawal of blood from the surface layers. The further observation made by Bazett and McGlone that the skin gradient is less than the fat gradient in cold conditions and greater in warm is curious but susceptible of rational explanation. If we accept the findings of Lomholt (64) the thermal conductivity of the skin taken from the lean rump of corpses is 0.00046–52 cals/cm^3 sec°C., while water in the same units comes out at 0.0014–5 (65). Combining these results with those of Borra (13) indicating a sudden, very large increase in the water content of the skin in warm surroundings amounting according to Schade and Marchionini (12) to nothing less than a total change in nature of the skin from lipogel to hydrogel, we see that skin conductivity in warm conditions must be greater than in cold. Now remembering that the increased vascularity of the sub-dermal layers under warm conditions will raise the temperature inside the skin above what it would otherwise be, while its increased conductivity will enable the outside to approximate more nearly to that of the surrounding air, we get a clear picture of why the skin gradient should become steeper in warm surroundings. The temperatures called *warm* by Bazett and McGlone are identical with those at which Borra found this great increase in insensible perspiration. Incidentally support is afforded to Barr and Du Bois' (66) idea that the sharp bend of temperature gradient may not be at the skin in fever.

This work renders pertinent the enquiry whether these gradients may not be affected by the *composition* of fat as well as its quantity. That food may affect the composition of fat has been known for some time, Ellis and his coadjutors (67) have been working for some years on this phenomenon in pigs and Cathcart and Cuthbertson (68) quote numerous observations of a like nature in man and show, furthermore, that even apart from these variations there exist definite local differences in composition at different parts of the body. The data are scanty but as far as they go seem to indicate that no great variation in gradient is to be expected with varying composition. The thermal conductivity of olive oil, castor oil and poppy seed oil for which fairly reliable data are available (69) all come out in the neighbourhood of 0.0004 cals/

cm^3 sec °C. (= 0.0017 watts/cm^3 sec °C.) at 4°C. Of these poppy seed oil has an iodine number round about that found by Cathcart and Cuthbertson for liver fat, while that of the other two oils is rather higher than the values found for other body fats.

Of interest from the above point of view and even more in a general way, especially to the farmer, are the observations of Zummo (70). He finds that the iodine number of the fat laid down by albino rats depends upon the temperature of their surroundings in such manner that the fat of rats kept at 0°C. has an iodine number very distinctly smaller than that of the fat of others kept at 30°C. A period of eight days sufficed to show this effect and it is independent of the food. It seems to call for a more thorough investigation of the thermal conductivities of fats, more especially with an eye to possible differences between saturated and unsaturated types. One envisages the possibility of another mode of heat regulation operating more slowly, perhaps accounting for some phenomena of seasonal change in heat elimination.

Naturally any asymmetric deposition of subcutaneous fat or unequal conductivity of the skin, whatever its cause, must affect the surface temperature and there appears no reason to ascribe the variations observed by Cotte (71) at symmetric points in the ankles to any deeper or more fundamental cause; indeed from the information supplied it is difficult to see how changes due to varying air currents or unequal external insulation were avoided.

Swift (72) states that the onset of shivering is delayed by a thick layer of subcutaneous fat. This may account for the greater ease with which women accustom themselves to the conditions of employment as artists' models. Swift's further observation is curious however; he finds that the stimulus to shivering is not a fall in body temperature, but the attainment of a skin temperature of about 19°C. (cf. Bazett and McGlone p. 440). It seems singular that the subject with a thick subcutaneous fat layer should respond to the same temperature as a thinner one unless the stimulus is nervous, in which case it is no less difficult to understand why this thick layer of fat acts in the way it apparently does in delaying the onset of shivering. A fuller account of this work will be awaited with interest.

Of equal importance with the temperature gradient in the superficial layers is the surface area of the body when heat elimination is in question.

The surface law has come in for severe criticism during the last decade. In detail it cannot of course be true, but in this matter even Rubner himself agrees (9, p. 289). Showing a perfect appreciation of the ob-

jections based on the failure, in reckoning geometric surface, to take into proper account the fact that the effective radiating surface is never quite the same as the geometrical one, see Bohnenkamp (1). The difference between the two is a function of body shape and the position selected and in human beings often anything but negligible even when referred to a standard position. Hence two distinct surfaces come into the computation, the geometrical which may perhaps be considered operative for heat loss by conduction, convection and evaporation whether the temperature be above or below that of skin change of Schade and Marchionini (12) and a special surface, equal according to Bohnenkamp and Pasquay (l.c.) to 4π times the square of the electrical capacity, which is operative for that portion of the heat which is radiated from the body. The difference between the two arises from the fact that certain surfaces of the body radiate towards one another, either entirely or in part. Geometrical surface must also affect the metabolism corresponding to the cutaneous respiration, see Shaw and Messer (73).

Approximately contemporaneous with the above work is that of Kleiber (74) who ably discusses the theoretical implications of the surface law from all points of view, and gives in addition a good bibliography. His arguments in favour of relating metabolism measurements to a higher power of the body weight than $\frac{2}{3}$ depending as they do on considerations of the intensity of blood flow to the tissues connect up with Purdy, Johnson and Sheard's work noticed earlier.

A principal difficulty in reaching a settlement of this vexed question has been the lack of sufficiently accurate means of determining what the surface area of an animal is, even in a standard position. The writer has recently published (75) an account of a new method of attacking the problem by mathematical means with an accuracy of ± 2 per cent.

The possibility that the relative importance of the two operative surfaces may change from time to time is suggested by the work of Borra, of Schade and Marchionini, and of Shaw and Messer already spoken of.

It may well be that the question of the surface law will be absorbed eventually in the larger one of the application of another general law discovered as a special case by Rubner, namely, that of uniform metabolic power of vertebrate tissues excluding man. This law has recently been given much wider scope by Bauer (76) who finds the quantity

$$\frac{\text{basal metabolism} \times \text{duration of life (days)}}{\text{body weight (kgm.)}}$$

to be approximately constant for each class of animal, but to increase steadily from 5.8×10 in coelenterata, through echinoderms, worms, molluscs, arthropods and vertebrates to 9.3×10^5 in man.

While the physical regulation already begins to lose the definiteness of its boundaries and shade off into the chemical at numerous points of contact the distinction remains useful. The effects already mentioned as observed by Gessler and by Benedict and Ritzman, which amount to a time effect on basal metabolism are hardly capable of classification as yet while Gessler's point that it is not possible to differentiate the chemical from the physical regulation by means of temperature considerations only, since chemical regulation *alone* is insufficient in amount to account for the maintenance of constant temperature in very cold surroundings, is not to be forgotten. One must allow the existence of variation of heat loss as well.

Physical regulation of body temperature proceeds by conduction, convection, radiation and evaporation. The first two of these may be considered from the internal and from the external standpoint while radiation and evaporation both operate externally only as far as heat elimination is concerned.

Conduction from the internal point of view has already been considered in some degree in connection with the maintenance of constant temperature in the body. Consideration of temperature gradients, depending in so far as conduction is operative on fat mainly, leads to two different end points according as the discussion is pursued regionally, leading to temperature topography; or generally, leading to considerations of "mean skin temperature" specific gravity, and basal metabolism. The same dual path is available also in considering convection, radiation, etc., and Bohnenkamp and his collaborators aim to show that the integration of observed effects along the regional path equals those obtained by following the general.

The gradient is naturally a function of the internal temperature as well as of the external and hence Stigler (62) suggests that in the ability to tolerate an exogenous hyperthermia for a long period the rodent is actually provided with a new form of heat regulation since by allowing its body temperature to rise it increases the loss of heat by conduction from a negative quantity to nil. At the same time the loss by convection, radiation and evaporation is also raised.

Clearly the rate of heat loss by conduction to the surface is also modified by the vascularity of the surface layers, so that such effects of internal convection as are mentioned later must affect the conduction quota.

It has been pointed out by Barbour that the obvious fact that the danger from overheating is normally from within while that from overcooling is normally from without finds a physiological expression in the situation of Ruffini's corpuscles in deeper layers than Krause's bulbs.

Very high external temperature causes vaso-constriction according to Berti (77) recently confirmed by Pickering (77a) and this serves a useful purpose in reducing the temperature gradient, thus minimising inward conduction apart from its more obvious purpose in lessening internal convection.

The amount of fat clearly cannot be varied suddenly but the internal temperature gradient and therefore the rate of heat loss may be so, not only by change in temperature difference but by alteration in effective thermal conductivity by vaso-motor means, or otherwise.

Since the subcutaneous fat acts as an insulator the temperature gradient and therefore, under like conditions, the rate of elimination of heat by conduction must vary at different parts of the body since the fat layer is not equally distributed. In considerable cold, moreover, the extremities may become supercooled and the circulation be appreciably retarded, while Kirk (78) has shown that a change in the opposite direction takes place in sleep and anesthesia. Both these effects have an obvious influence on the regional output of heat by conduction through the surface layers.

The order of difference likely to be made in the amount of heat transferred through the skin at different parts from these causes together with internal convection differences, is apparent enough from the observations of Bohnenkamp and Ernst (1 tables 4 and 5). The differences between the tops and soles of the feet are specially noticeable both in themselves and as between the different subjects. Although the observations are actually of heat radiated the skin temperature on which this depends is affected by conduction.

In view of the above facts, the question arises as to the possible percentage accuracy of the radiation loss determination by Bohnenkamp and Pasquay (1) (*Mitt V*) in which the mean radiating temperature of the skin is determined from the specific gravity (sic). This computation gives T_S in the equation—

Energy loss by radiation =

$$\sigma(T_S^4 - T_E^4)\ 86400\ S_R$$

where σ is the universal radiation constant, S_R the "effective radiating surface" and T_S and T_E the absolute temperatures of the skin and en-

vironment respectively. From the graph, p. 151 of the above paper, it does not appear that the investigators can get nearer to the mean skin temperature than ± 0.3°C. working from a specific gravity itself affected with an error ± 1 per cent (l.c.) whence it is easy to compute that the error in the energy loss by radiation, working as they do with a room temperature 25°, amounts to about ± 6 per cent, increasing rapidly as the room temperature is raised. Hence it would appear desirable in using the method to employ the lowest room temperature found feasible by Bohnenkamp and Ernst (l.c.) namely 23°. Even then from Bohnenkamp and Pasquay's results (1) *Mitt. III* it appears not improbable that a corresponding reduction in skin temperature might render this advantage almost nugatory. On the other hand the lower the temperature the less trust can be placed in the mean skin temperature deduced from the specific gravity, since abnormal cooling of the extremities may come in.

In considering the values quoted in tables 4 and 5 of Bohnenkamp and Ernst (l.c.) it is perhaps well to remember that no *general* deduction as to *relative* values can legitimately be drawn from them; since the difference between the 4th powers of two temperatures is a function of the absolute values of those temperatures. Cobet and others (79) find appreciable personal differences in this matter and aver that constancy of temperature is not reached at the skin for ¾ hour after the clothing is shed. A trained subject cooled the legs more and the chest the same as an untrained one hence the training was effective in producing a better use of the heat regulating mechanism.

A fall of external temperature should intensify the difference between skin temperatures at isolated spots in so far as these are dependent on conduction through the superficial fat layers. The temperature topography of the skin however depends also on the situation of exothermic chemical activity in the body. This has been a subject of controversy (see Barbour's review). The recent observations of Benedict and Fox (16) on pythons speak emphatically in favour of this dependence since they found a noteworthy increase of temperature, amounting to several degrees, at the bulge in the reptile's body when it was digesting a meal. The possibility of this being to some extent under control is opened up by their latest work on the incubating python (80).

Barr and DuBois (66) state that skin temperature is not necessarily a criterion of slow or rapid heat elimination since the sharp bend of temperature gradient may not be at the skin. Bohnenkamp and Pasquay (1) *Mitt. III and V*, claim that heat radiated is very definitely

a function of the fourth power of the absolute skin temperature and if, as they claim, this is the greatest fraction of the total heat eliminated, which must of course reach the surface by conduction and convection combined; we might anticipate a like dependence of the total heat elimination on the skin temperature. Barr and DuBois find that this is not so, they state (p. 644) that when the internal and external temperatures are fixed, whether the skin temperature is high or low ". . . in both cases the individual produces within his body the same amount of heat per hour and eliminates the same amount per unit surface." The only feasible explanation of this paradox is that as the amount of heat radiated increases the amount passing off as insensible perspiration decreases. This we shall see later is apparently the case, at any rate when conduction and convection losses are reckoned in with radiation loss. If this is so however it appears that present ideas in the matter of insensible perspiration in relation to basal metabolism will have to be modified.

Bohnenkamp and Pasquay (1) *Mitt. V*, give weighty reasons for thinking that the well-known difference in basal metabolism between males and females of many different species, including man, depend in the first place on skin temperature difference due to greater thickness of subcutaneous fat in the female. This extra fat lowers the specific gravity, that of women being found to be distinctly less than that of men. Arguing from this they are able to account satisfactorily for (a) the fall of basal metabolism with age, (b) the greater approximation of the sexes in this regard as age advances. Also it seems to explain the greater ability of women as swimmers and certain peculiarities of the metabolism of pachyderms and cetacea. One noteworthy point is that the reduction in skin temperature due to fat, in a fat man, actually makes a greater difference in the metabolism in a negative direction than does the consequent areal increase in a positive direction.

How far the determination of metabolism by specific gravity and electrical capacity measurements will justify itself must be left to the test of time, but one cannot but feel that some experiments on the test objects used by Bohnenkamp and Schmäh (1) with these filled with water at, and perhaps also slightly above, body temperature would have afforded a valuable check on the performance of the bolometers. Furthermore, since the volume of a human being appears to be from 60 to 90 litres and the total volume of the lungs, mouth, etc., is about 4 litres (81) it is questionable whether the error involved in correcting a water immersion experiment for the air estimated to be in lungs, etc.,

would be so much greater than the 0.7 to 1.0 per cent found for the complicated gas immersion test employed. The simplification would be a great advantage.

Mark (82) and later Wieland (83) have shown that the subcutaneous fat layer always acts as an insulator causing the fat man to make an *earlier* call upon his physical regulation than does his thinner brother.

The recent findings of de Bruin (84) that the gas exchange of fat children is within the normal limit of variation is reasonable in view of the facts mentioned on page 446, especially as some of the subjects were noted as being too long for their age.

Even with the naked body, at rest, the question of *external* conduction is a complicated one, owing to the fact that as soon as the air in contact with the body has become warmed a little convection currents are inevitably set up. On the other hand it is equally true that no such convection current can start before either some heat has been passed out from the body by simple conduction or some water has been evaporated, reducing the density of the air with which it is admixed. Usually both processes go on together.

Probably heat loss by conduction, externally, is more dependent on *geometrical* surface than any other process with which we are concerned here, though even so the axillar and perineal regions are exceptional.

Very pertinent to this question is the investigation of McGlone and Bazett (85) on the temperature of the air in contact with the skin. They found that up to a distance of 1 mm. from the skin the air in contact therewith was less than 1°C. below it in temperature under comfortable or warm conditions. This layer was reduced to 0.5 mm. in cold air. Air movement affects this to a marked degree, and hair or fur provides what they felicitously term a "private climate." Except for this interference the law of decrease of temperature is exponential.

Clothing or a coat of hair or fur greatly reduces both conduction and convection externally and hence, except for the relation to evaporation loss mentioned, would greatly affect metabolism. There are many extant observations of the result of removing a covering, natural or otherwise. A. Giaja (86) claims an increase of 78 per cent in geese and 54 per cent in hens as the result of removal of feathers. He claims that the feathers in geese are not passive insulation but active physiological agents used for heat regulation by variation of elimination. They afford better protection the colder the exterior and the effect is not noticeably affected by wind. Turkeys have less thick feathers and the heat regulation is partly chemical, that is, the critical temperature is

higher. Benedict and Benedict (87) found no difference in the amount of insensible perspiration and therefore presumably metabolism as between the clothed and nude condition in an artist's model, while Kriss (88) finds the insensible perspiration actually increased by the possession of a winter coat in cattle, presumably as the result of the balance mechanism previously mentioned. The heat production of the shorn animal was however greater according to Forbes (89) if his figures refer to the same animal, as appears to be the case although his communication is now seven years old. Ritzman and Benedict (90) find, with sheep, an effect of shearing fully comparable with that of Giaja on geese. The vexed question therefore as to whether clothing, or natural coat, has any insulating action the effect of which is apparent in heat measurements may probably be considered as answered in the affirmative.

Internal convection currents play an important rôle. Clearly, the steep temperature gradient in the ears of elephants and rabbits although resulting in a heavy loss by conduction and probably evaporation is primarily *due* to the high vascularity and blood flow and hence if these are affected in any way there will be a change at the top of the gradient causing this to adjust itself and the rate of heat loss will be changed, and similarly in other cases (but see Grant, R. T., et al. Heart, xvi, 69, 1932).

Heat loss by internal convection takes place also, physically speaking, by the vaso-constriction observed by Berti (l.c.) at high external temperatures in that this causes a loss to the body of the extra, unwanted heat which would otherwise be carried in from the surface by the blood stream.

On cooling the body the blood sinks below the subcutaneous fat and Krogh (91) has shown that on warming again acceleration of the blood stream by the widening of the capillaries, arterioles and veins can considerably augment the flow without any actual increase in number of capillaries, but that in all probability there is also an increase in the number of these in active use. Physically there are several factors at work here. An opening of the capillaries must inevitably cause a fall in pressure difference at their ends, i.e., in driving pressure causing blood to flow through them, and this in its turn must result in a rearrangement of pressure gradients in the vascular system generally so that the quantity of blood flowing is increased by reduction of friction head in comparison with other parallel paths. The process is further assisted, presumably, by the reduction of viscosity due to the greater water content of the blood under these conditions discovered by Barbour and since

confirmed by the experiments of Marx, Barcroft and others (for numerous references see Gessler).[2]

The destination of the blood which is driven from the surface in cold surroundings is uncertain. Gessler quotes authorities for the statement that it goes to the muscles and abdominal organs where extra heat is generated for chemical regulation and oxygen is therefore required. The work of Barcroft, which he has summarized recently (92), goes to show that the blood used need not necessarily be actively employed the while, but may be passively stored in the liver and especially in the spleen and discharged thence by a nervous mechanism as required.

Meanwhile warmth is not the only means of inducing a change in the peripheral blood flow. Various drugs, adrenaline, nitrites, etc., occur to one immediately, but Cobet and von Haebler (93) have lately shown that this can be done by simple immersion of the body in CO_2 gas in the cold. Under these circumstances the peripheral blood flow is notably increased, while the CO_2 absorption by the skin has no significant effect on the metabolism. It appears to offer a new field of work, of somewhat uncertain promise perhaps, on the problems of heat regulation as affected by internal convection.

External convection is of two kinds—free and forced. In free convection the surrounding air is at rest except for the gravity air currents set up by the warmth of the body. The physical definition of forced convection stipulates that the ambient fluid shall move owing to external causes with such velocity that the free convection currents set up may be neglected. We see then that under laboratory conditions, without clothing, and at rest in a room without fans, a close approach to free convection conditions is made, while in normal life a man or an animal doubtless approximates more or less nearly to forced convection conditions. In the case of man and some animals the problem is further complicated by the continuous giving off water vapour either by sensible or insensible perspiration. This effect *will not be purely additive*, causing increase in the gravity current in free convection by admixing a greater or lesser quantity of water vapour of density = 9 with air of density = 14.7, since the actual process of evaporation entails a lowering of surface temperature, otherwise it could not perform its function in temperature regulation. Hence, at first sight, there appears no *absolute certainty* that the convection current need be increased at all by evaporation since circumstances are easily imaginable in which the

[2] Such an effect of viscosity has been rendered almost certain by E. M. Landis since the above was written, see Amer. Journ. Physiol., 1933, ciii, 432.

surface might even be cooled below the room temperature, and anyhow there is no guarantee that the extra heat outflow due to the steeper gradient will do more than cover the cost in latent heat of producing the vapour. What makes it certain that an appreciable increase in convection will take place is the enormous relative volume of vapour generated, a cubic millimetre of water producing more than a cubic centimetre of vapour of density = 9. In short all we appear to be justified in stating is that the presence of perspiration whether sensible or insensible will augment algebraically, to some indefinite extent, any effect which may be deduced for free convection.

A short treatment of both free and forced convection in its general physical aspects is given, together with a considerable bibliography by Rice (94). It is well brought out by Griffiths and Davies (95) that geometrical form and even orientation are not without influence on the quantity of heat lost by convection. Thus an oblong plate in a vertical plane cools more quickly when its length is horizontal and its width vertical than in the reverse conditions. Hence only a rough idea of the amount of heat which may be expected to be lost from the body by free convection in air can be hoped for, but as it happens this admittedly rough figure is of considerable interest, since it is larger by a great deal than that obtained for it by Rubner (8) *by difference*, and again last year by Bohnenkamp and Pasquay (1) *mitt. V* also *by difference*. A vertical cylinder of area equal to that of the subject W of the latter authors maintained 6.5°C. above its surroundings, corresponding to the difference between room and skin temperature given by them for a room temperature of 24°C. loses between 600 and 700 Cals./day by free convection whether we use the formula of Rice or that of Griffiths and Davies, while the original authors assume that 100 Cals./day is an *ample* allowance for heat loss on this account for the man in question. The work of Griffiths and Davies suggests that the difference in form between a man and a vertical cylinder is *in no way adequate* to explain this large difference, even when the man is standing on a floor instead of being suspended. Evaporation it is clear, must intensify the disparity between the figures. It is moreover impossible to think that when a person is normally still, there will not be *some* movement which will assist heat loss. The question has recently been investigated in some detail by Aldrich (96) and his results obtained with *normally fed* subjects showed the total heat radiated did not exceed and was usually about 80 per cent of the *basal* metabolism, or 60 per cent of the total radiation and convection loss and Büttner's figures (96a) fully confirm this. It is

much more in accordance with what one would expect. Since orientation is of importance in considering convected heat elimination it is desirable that some difference should be made between the standing and the lying position when abnormal or young human beings are considered. For the grown man quoted above there is but little difference, considering the convection from an equi-areal cylinder, but since in this position the thickness of the cylinder enters into the computation inversely as its fourth root, while in the vertical position convection is independent of diameter the bearing of this on observations in obesity or of small children must be remembered. The heat loss in the vertical position is moreover proportional to a higher power of the temperature difference than in the horizontal.

Forced convection has hardly been studied except in regard to cases where free convection is negligible, consequently one is at a considerable disadvantage in discussing the effect of the smaller movements on heat loss. If one employs the formula given by Rice (l.c.), the heat loss by forced convection from a horizontal cylinder of the area of the man previously mentioned in an air stream of 300 cm./sec. would be about 5 times the free convection loss but this is to make several unwarrantable assumptions when the body is substituted for the cylinder. The air blast will reduce the skin temperature slightly, as shown by Benedict and Parmenter (97), greater differences have been observed by Strauss and Schwarz (97a), and this will reduce the convected heat. The *potential* rate of evaporation will be increased but the skin will under most circumstances reduce this by supplying too little water. Between them these factors may appreciably reduce the above multiplier. Even at the very low air velocity of 30 cm./sec. the computed convection comes out rather higher than free convection. As under such circumstances the above limiting factors cannot operate to any great extent and it seems likely that some sort of vectorial summation of effects of the free and forced convection is to be anticipated, it would appear justifiable to assume that *slight* movement may augment heat loss by convection to an extent varying with the amount of movement but probably less than doubling the amount of it.

This estimate agrees very well with the practical observations of Houghton, Teague, Miller and Yant (98) who found the total heat loss from the body by convection and radiation (including conduction) increased by about 50 per cent in an air stream of 385 ft./min. (= 195 cm./sec.) although the relative humidity was 95 per cent. Radiation cannot have been increased, since skin temperature must have fallen

slightly according to Benedict and Parmenter (l.c.); we conclude therefore that the increase must be due to convection. At the above air speed the computed forced convection is approximately four times the free. Hence the 3–5 per cent estimated by Bohnenkamp and Pasquay for free convection might, if correct, become 12–20 per cent; an *increase* of 9–15 per cent as against 50 per cent observed. Aldrich found a comparable increase with his metal cylinder. In the above reasoning no account is taken of the skin cooling which would be present to some small extent and widen the discrepancy to the disadvantage of radiation owing to the fourth power of the temperature being involved.

Benedict and Parmenter (l.c.) also have an interesting observation on an artist's model who remained naked in the laboratory for an hour before the experiment and was then able to increase her metabolism by over 600 per cent by walking on a treadmill up a gradient of about 1 in 6, without thereby increasing her skin temperature at all. Of course a considerable amount of this increase will be accounted for by extra evaporation from the skin and lungs but to account for the whole of it in this way demands that this quota be multiplied by 24 as a mere result of walking. The skin temperature was, it is true, in the range of maximum variation of insensible perspiration of Borra, but the skin temperature was, if anything, lower. If however one may assume a moderate increase in Bohnenkamp and Pasquay's estimate the above figure may be largely reduced and for this Aldrich's results afford every justification.

From the physiological point of view the general effect of wind and weather on basal metabolism has been investigated by Benedict and Crofts (99) who find it *unaffected*. Benedict and Benedict (100) find the same result for the insensible perspiration alone. Houghton, Teague, Miller and Yant (l.c.) confirm Benedict and Crofts' results on the whole but they find that if the temperature is below 12°C. the wind has an effect which increases as the temperature falls (see also ref. 97a). As farm animals are usually kept at lower temperatures than this, at any rate for the greater part of the year, it is likely that the subject would repay further study from the agricultural side. Forbes, Braman and Kriss (100a) confirm the above results on cattle, but worked at 21.8°C.

Bohnenkamp and his collaborators rightly emphasize the essential difference between the geometric surface, and their special "operative surface for radiation." If, however, the energy convected and conducted from the body is considerably more than 3–5 per cent of the whole, it raises very appreciably the general importance of geometric rather than radiating surface. This is further increased when farm animals are in question,

since we have here, almost certainly, a preponderance of forced convection under natural conditions; and apart from this, the ratio loss by convection:loss by radiation will be increased by the low surrounding temperature, the radiation loss varying as the cube of the absolute temperature for the same temperature difference while the convection remains unchanged or practically so. It may be remarked in passing that under Bohnenkamp's conditions of complete nudity at 24°C. and medium humidity a maximal rôle is thrown on radiation for keeping up a proper heat loss.

Heat loss by radiation is in the nature of things wholly external. It consists of long wave infra-red rays corresponding to a skin temperature of about 32.5°C. ($= \lambda_{max}\ 9.44\ \mu$) (1) *Mitt. III*, at normal body temperature. Bohnenkamp and his co-workers (1) have shown that this radiation follows all the ordinary physical laws and that therefore when two antiposed surfaces radiate directly on to one another there is at that place no net radiation loss whatever. In consequence the measurement of this fraction of the heat loss *per unit surface area* is meaningless when the surface referred to is the *geometrical* surface of the body. Bohnenkamp and Pasquay (l.c.) introduce what they call the "operative surface for radiation loss." An awkward expression which in the writer's opinion might with grace and convenience be renamed the "Bohnenkamp-surface."

This surface they give reasons for supposing equals numerically to $4\ \pi \times$ the square of the electrical capacity. Hence it varies with the position of the body, especially as to whether it is bunched up or extended. In certain pathological cases this differed so materially from the normal as to account for the very abnormal metabolisms recorded for these subjects when computed in the ordinary way.

It may be noted however that the connection between the Bohnenkamp-surface and the experimentally determined electrical capacity depends on the *assumption* that the surface of a human being is a perfect electrical conductor, insulated, and not containing within itself any source of E.M.F. Insulation can be more or less achieved but neither of the other conditions is strictly true as may be seen from the experiments of Purdy, Johnson, and Sheard (see p. 434) and those of Waller (101). The first named workers selected their position for measurement on account of the small number of sweat glands in this region and showed that the phenomena were unconnected with sweat, but probably with colloid changes in the skin as earlier opined by Waller. This may now perhaps be interpreted in terms of the results achieved by

Schade and Marchionini (see p. 440). The measurement of the Bohnenkamp-surface by the electrical capacity in the manner of Bohnenkamp and Pasquay may therefore be affected by E.M.F. change which is itself affected by blood flow and so correlated with basal metabolism. Hence the measurement of radiation loss per unit of Bohnenkamp-surface *may* be about as indefinite really as measuring sugar by number of lumps; it may, however, also turn out to share the common convenience of this enumeration of slipshod units.

The emissivity of the skin is high, Stewart (102) found it about equal to that of lamp black while Cobet and Bramigk (103) have recently found a still higher figure confirmed by unpublished observations of Rubner and others of Abbot cited by Benedict and Parmenter (97). We must note carefully, however, that this applies solely to the radiation about 9.44 μ in the infra-red. Martin (104) has demonstrated clearly that when we are regarding the body as the *receiver of radiation* generally the case is very different and the eye is not in error when it tells us that white skin is a better reflector and so a poorer radiator than black and that this applies not only to the light but to some considerable part of the heat rays as well. Keller (105) has investigated the matter more recently and has shown that when heat rays of 9.44 μ are used blackened skin is not more absorbent than unblackened. The emissive power of the skin is unchanged by smearing it with unguents (106).

The energy the body loses by radiation is really the difference between that emitted and that received. That this must be so is more or less obvious but its demonstration is due to Bohnenkamp and his collaborators especially Pasquay.

The amount radiated away per unit time has been shown by these experimenters to follow the Stefan-Boltzmann relation, i.e.,

$$J = \sigma(T_S^4 - T_E^4)\, S_R \qquad \text{(See p. 444)}$$

Barbour's point that the radiation varies with the peripheral blood flow follows of course from this since T_S is changed, as also does the observation of Phillip (107) that radiation is increased by massage since the peripheral blood flow is altered. His other observation on the rapidity of fall of radiation after disrobing is clearly contained in the equation and accords with the earlier observations of Stewart (102) who traced it to reflex vaso-constriction beginning immediately on exposure. This of course results in lowering of surface temperature and a much more rapid fall of radiation in accordance with the law above.

Martin's observations render it quite clear that a real difference does

exist between white and coloured races as regards absorption of heat radiation from without. Of the heat energy of the noonday sun in London in summer an average blonde skin absorbed only 57 per cent, a semitic brunette 65 per cent, a Hindu 78 per cent and a negro 84 per cent. The matter has been further investigated by Angus and Taylor (108) and by Meyer and Kirchoff (109). Both these workers agree more or less in finding that the longer waves pass the outer layer of the skin and are absorbed somewhere below this, especially by melanin. Plotnikow and Mibayashi (110) have recently considered the scattering of such rays in the tissues and conclude for a lower penetration of body tissues by such radiation than has previously been considered probable. Hence, on the whole incoming radiant heat appears to deliver its energy to the body somewhere in the surface layers.[3] The possibility of the receipt of small quantities of energy by the body from certain hitherto unsuspected sources seems to be opened up by the work of Wiggen and Orias (111) who have induced a hyperthermia with wireless waves.

The amount of heat radiated depends, theoretically, not only on skin temperature but also on the room temperature. This latter is difficult to define properly since the actual radiation takes place only to a small extent to the air and largely to solid objects in the room, walls, ceilings, floors, etc. Consequently, the equation may fail if T_R is taken as the air temperature. The matter never seems to have received adequate attention, it is just touched upon by Dufton (112).

In the *receipt* of energy in the form of radiation the Bohnenkamp-surface applies only to the somewhat rare case when the body is receiving radiation from the more or less uniformly heated walls, ceiling and floor of a room. Normally, radiation is received unilaterally from a source definitely oriented at any particular time with regard to it. The gain from sunlight involves the solar constant, the projected area of insolation, the state of the atmosphere and the altitude of the sun. Dorno's figures (45) agree within reasonable limits of error with what might be expected from the ordinary law of absorption of radiation by a medium, viz., $I_x = I_o e^{-kx}$ allowing for the thickness of air to be penetrated. Clearly in reckoning the gain, allowance must be made for the amount lost by scattered reflection as in Martin's experiments. As practically all the heat waves received from the sun are of wave length $< 2\mu$ it is plain that this difference between the races is of importance, and *mirabile dictu* that as far as this matter is concerned the white man

[3] Cf. F. Rücker, Pflüger's Arch., 1933, ccxxxi, 742.

appears to be better fitted to withstand the heat of the tropics than the Negro. The experiments of Marsh (113) are interesting in this regard. He has found that albino rats are able to stand a much higher temperature than black ones in Persia. His further observation that after watering, the albino rats were only able to stand a little more while the black ones were brought up to the level of the white is easily explainable. Aschkinass (114) has shown and his observations have been very fully confirmed recently by Dreisch (115) that in the region of the infra-red in which the greater part of the solar heat radiation lies, i.e., from about 1–2 μ, water acts as a powerful absorber (there are four maxima included from 0.995–1.97 μ), so that as far as these rays are concerned the white rat is turned into a black one by watering. Naturally the black rat would be able to stand more heat after watering owing to the cooling effect of evaporation so that the *appearance* may easily be that the black rat's resistance to temperature is brought up to that of the white.

To what then is the greater ability of the coloured man to withstand heat to be ascribed?—The observations of Meyer and Kirchoff already mentioned confirm earlier observations of Hill (116) pointing to melanin as the great absorber of solar heat. This is in the surface layers and it may be that by rapid heating it brings the sweat glands into play earlier. According to Stigler (117) the body temperatures of Negroes is the same as that of whites and he ascribes their superiority to more rapid recovery after overheating. It seems also possible that the incident radiation may be radiated again directly as waves of $\lambda_{max} = 9.44$ as the result of the increase in skin temperature ensuing as a direct result of insolation, though Stigler states definitely that the pigment is not the protection. In any case he observes that even negroes and tropical animals avoid exposure to the full sun if possible.

It seems likely however that the inferiority of the white man in toleration of sunlight may rest on something quite different. Bachem and Reed (118) find that the visible and near infra-red rays down to 1.4 μ are strongly absorbed by the blood of the chorium and subcutaneous layer. The far infra-red on the other hand has very little penetrating power and is most strongly absorbed in the epidermis. In Negroes one would expect that also short wave heat radiation would be retained by the melanin instead of passing to the blood as in whites. Both infra-red and ultra-violet rays have been shown to have various chemical effects in the body among these the reduction of blood sugar demonstrated by Lucca and Reviglio (119) and the immediate reduction in pH of the blood of tissues underlying skin exposed to sunshine men-

tioned by Laurens (120) appear by no means impossible precursors of untoward effects. Moreover Gigon and Noverraz (121) have found an appreciable effect of carbohydrates on permeability to infra-red radiation while Franz (122) has shown an effect of water content of skin on absorption in poikilotherms which might well have been anticipated in view of the strong absorption bands. In the chemical laboratory Palit and Dhar (123) have shown an ability of sunlight to promote oxidation of glycogen and other carbohydrates, amino-acids and fats. All these observations lead one to consider very carefully the possibility that the solar heat rays may set up irregular chemical changes or modify regular ones in the organism in a way from which the Negro is protected by the outer coat of melanin with which he is endowed. The experiments of Küstner (124) show that the infra-red rays are capable of accelerating certain bodily changes and this may well add fuel to the fire which is already too hot. The antagonizing effect of the shorter wave lengths observed would be ineffective below the epidermis as these would be filtered out, indeed Reviglio (125) has found that such antagonisms are present in the epidermis but not below. *Per contra* the results obtained during the last few months independently by Schear (126), Nasset (127) and Hodgson (128) pointing to a close positive correlation between blood sugar level and metabolism under most varying conditions. Thus strong insolation might be expected to produce *reduced* metabolism via reduced blood sugar (cf. Radsma (18) (19)).

It is very likely that the sweat itself, poured out upon the surface of the body, has a protective action owing to its ability to absorb solar heat rays ($\lambda = 1\text{–}2\ \mu$) as well as the more obvious one due to its high latent heat of evaporation.

Incidentally it may be remarked that according to Goldhammer (129) water exhibits also a strong absorption band in the region $\lambda = 9.44\ \mu$ so intimately connected with the natural radiation from the body. Hence it should be possible to obtain further confirmation of the quantity emitted on this wave length by observing with thermopiles through water films.

The fourth mode of heat elimination, evaporation of water from the surface of the skin, constitutes the chief factor in what is commonly known as the physical regulation of body temperature. In this case approach from the physical side yields results which have apparently far less relation to the physiological facts than in the other cases discussed. The physical facts themselves, indeed, are not known with any great exactitude although such problems have continued to fascinate

workers ever since Apjohn's attempt to find a theoretical formula for the lowering of temperature at the wet bulb of a hygrometer. It is known (130) that with a certain amount of wind the evaporation per unit area from a flat flush surface is approximately proportional to the vapour pressure and to the square root of the wind velocity. It is also considerably affected by the area itself, the geometric form of the surface, its presentation to the wind, etc.

Gessler emphasizes that regulation by evaporation is developed to a far higher degree in man than in animals owing to the frequent absence or partial absence of sweat glands in the latter. These however often have other evaporatory mechanisms such as polypnea which is common, or even the peculiar rodent type of Stigler (22) in which a nasal secretion is poured out over the surface of the body where it evaporates.

The total quantity of water normally evaporated from the body was investigated by Soderstrom and DuBois (131) in a historic paper, in which they estimated the secretion through the skin and air passages to *average* 29 gm./hr. corresponding to about 24 per cent of the total heat produced. Newburgh (132) has confirmed the above work but in two later publications in collaboration with Wiley (133) he finds this constancy less apparent. It holds between 24°–30°C. in the nude but increases above the latter temperature. Increase of relative humidity, they find, reduces both the total water elimination and the percentage which this forms of the whole, while clothing abolishes the effect of relative humidity. They now deny that the 24 per cent relation holds. In this Jores (134) is at one with them. He finds moreover that only 17.4 per cent of the increment of heat production due to specific dynamic action is accountable in the water vapour increment. Hence in experiments where this is involved there can be no linear connection with metabolism. Fat, humidity, and vaso-dilatation he found without effect, differing thus from Wiley and Newburgh and also from Houghten, Teague, Miller and Yant (l.c.) in regard to humidity, that is. Levine and Marples (135) find, by statistical investigations, a high positive correlation between the physiological mechanism of heat production and the insensible perspiration. Manchester (136) has shown that this connection only holds if the hydration of the body is normal, while Borra (13) attempts to express the amount of insensible perspiration by means of an empirical formula containing a constant factor and one depending on the sweat glands. Lazlo and Schurmeyer (137) have also found large deviations from the 24 per cent relation in pathological cases.

It is remarkable that all this evidence against the truth of what was

beginning to be regarded as an established principle should all have accumulated within approximately the past year. It is quite plain from the original work of Soderstrom and DuBois as well as from that of Benedict and Root (138) whose values have been elevated to the position of standards, that none of these investigators had any idea of providing more than an average figure for general use. The number of observations is however quite sufficient to render the question of correlation certain, quite apart from the ordinary physical considerations which should almost have sufficed for this. In Soderstrom and DuBois' work the individual percentages H_2O/O_2 for normal subjects vary from 20 to 34 per cent, while Benedict and Root's figures show a margin of error up to $\pm$ 50 per cent becoming less with increasing metabolism and insensible perspiration. It is apparent that the greatest circumspection must be exercised in passing from observations on insensible perspiration to conclusions about the exact level of basal metabolism. Cf. remarks p. 446. The results of Kuno (139) showing the partition of insensible perspiration according to site on the body surface are also of interest.

The nervous control of evaporation heat loss is much closer than that of other types, and the differences in heat output brought about by differences in the state of the water evaporating mechanism are greater than those brought about by differences in other factors unless it be forced convection.

Houghten and his collaborators (l.c.) show with considerable clarity that the evaporation from the clothed body is so exactly controlled, presumably by nervous mechanisms, that at any one *effective temperature* the sum of the heat losses by evaporation and by other paths is constant. (Effective temperature is a measure developed by Houghten and Yagloglou (140) which allows not only for physical factors but for the psychological factor of comfort.) How this comes about is not difficult to see, since if evaporation from the skin is reduced the temperature of the skin will rise and the radiation, conduction and convection factors be increased, Viale (141) having shown that under these circumstances the amount of moisture breathed out from the lungs is also reduced.

An interesting turn is given to the general question by Kayser's work already discussed (p. 437) and it is evident that it opens the door to a better understanding of critical temperature itself and points the way to an eventual fuller understanding of the approximate 24 per cent relation.

It remains to add that the water required for constant evaporation

from the surface comes from the blood and in vaso-dilatation the increased flow serves to prevent over concentration, indeed as has been mentioned, the blood actually becomes diluted when the temperature of the surroundings is increased; so that the well known idea that a white man, resident in the tropics, has his blood thinned thereby is not entirely a nursery tale. One may note *en passant* that the concentration of the blood in cold weather is no doubt an adjuvant to chemical regulation by supplying both O_2 and sugar at a higher concentration. The matter is discussed at length by both Barbour and Gessler.[4]

Lazlo (142) has shown that heat regulation during mechanical work proceeds to a much larger extent by water evaporation than is the case in rest (cf. Jores on specific dynamic action, p. 458), while Whitehouse (143) finds that the amount of sweat is dependent not only on the body temperature but also on the fact of the performance of work. This may be due to raising of the skin temperature above the limit of Borra by the vaso-dilatation and heat generation during work. Diathermy (144) also increases the amount of water evaporated presumably, for a like reason but not more than 20 per cent of the added heat (usually much less) is eliminated as water vapour.

As in convection so in evaporation one would imagine that an increase must be induced by wind or movement of the air. Further on general principles it might be expected that with a low relative humidity the cooling would be greater, that is, the evaporation would be greater, than when the relative humidity is high. Houghten and Yagloglou (140) confirm the first of these but find that for some reason under most atmospheric conditions the second statement singularly enough does not correspond to fact, the precise reverse being observed.

It is not easy to say how far the surface of the body is functional in heat loss by evaporation since form comes into the picture and evaporation from free surfaces of liquid of the form of the human body has not been investigated. It might be possible to do so at high (Turkish bath) temperatures but the experiments would otherwise present great difficulties. Almost certainly the relation of free evaporation from the body, when neither recumbent nor nude, to surface must be remote. On the other hand for a nude person moving about slowly the surface area must have a preponderating effect. Doubtless the only reason why any relation to surface is noticeable at all in the ordinary way is because of the preponderance of the radiation-convection loss. An interesting light

[4] See also Barbour, H. G. and B. F. Aydelotte, Amer. Journ. Physiol., 1933, civ, 127.

is shed on the question by the observations of Dr. F. G. Benedict which he spoke of in a lecture given at Cambridge October 13, 1932. He has found that in the case of cold blooded animals whose heat of metabolism appears to be wholly voided by evaporation of moisture and which are moreover normally fairly torpid—the metabolism is pretty accurately proportional to surface area (snakes were used so this could be determined with ease) provided the temperature is unchanged. Defenders of the "surface law" will regard this observation by its eminent opponent with some satisfaction though some vital action of the skin as imagined by Vasti (145) might also account for this.

Houghten, Teague, Miller and Yant (l.c.) find that with a very low relative humidity (20 per cent) and an effective temperature of 27.6° the evaporation loss becomes equal to the total loss while at higher temperatures it exceeds it making the radiation-convection loss negative by cooling the surface. The extreme observation of this kind was at 38.6° effective temperature when the evaporation loss was no less than 285 per cent of the total. When the humidity was 95 per cent of saturation the maximum evaporation loss was found to be 74 per cent at 32° effective temperature.

The possibility that relative humidity may affect not only the manner of heat loss but its actual amount has already been mentioned (p. 429) and Shaw and Messer (146) have found an increased gas exchange at high humidities, thus confirming the earlier findings in the same sense by Rubner. The work of Murschhauser and Hidding (147) however which is well known remains the only definite attempt to elucidate the matter as a problem by itself. They found that metabolism was increased by humidity in warm surroundings but decreased in cold. Their explanation rests on a rise of body temperature in the first case, and in the second on increased evaporation from the lungs.

BIBLIOGRAPHY

(1) Bohnenkamp, H. et al. Pflüger's Arch., 1931, ccxxviii, 40.
(2) Barbour, H. G. Physiol. Revs., 1921, i, 295.
(3) Gessler, H. Ergebn. Physiol., 1928, xxvi, 185.
(4) Stigler, R. In E. Mangold's Handbuch der Ernährung u. des Stoffwechsels der Landwirtschaftliche Nutztiere. 1932, iv, 1–135. J. Springer, Berlin.
(5) Otani, K. Proc. Jap. Physiol. Soc., Jap. Journ. Med. Sci., 1931, ii, 2, 7.
(6) Schliephake, E. Verh. Deutsch. Ges. inn. Med., 1928, 307.
(7) Bělehrádek, J. Biol. Rev., 1930, v, 301.
(8) Rubner, M. Die Gesetze des Energieverbrauches bei der Ernährung. Wien, 1902, p. 212.

(9) Rubner, M. Sitzungsb. Preuss. Ak. Wiss. Phys.-Math. Kl. H. 16/18, 1931, 272.
(10) Martin, C. J. Phil. Trans., 1903, cxcv, 1.
(11) Kolmer, W. Pflüger's Arch., 1929, ccxxi, 319.
(12) Schade, H. and A. Marchionini. Arch. f. Dermatol. u. Syphilis, 1928, cliv, 690.
(13) Borra, E. Arch. di Fisiol., 1930, xxviii, 490.
(14) Kayser, C. Ann. de Physiol., 1930, vi, 721.
(15) Hall, F. G. and R. W. Root. Biol. Bull., 1930, lviii, 52.
(16) Benedict, F. G. and E. L. Fox. Proc. Nat. Acad. Sci., 1931, xvii, 584.
(16a) Adolph, E. F. Biol. Bull., 1932, lxii, 112.
(17) Du Bois, E. F. Basal metabolism in health and disease. New York, 1924, p. 332.
(18) Radsma, W. Geneeskund. Tijdsch. Ned. Ind., 1930, lxx, 786.
(19) Radsma, W. and G. M. Streef. Arch. Néer. Physiol., 1932, xvii, 97.
(20) Maresh, F. Amer. Journ. Physiol., 1931, xcvii, 544.
(21) Hashimoto, M. Journ. Orient. Med., 1930, 12.
(22) Stigler, R. Arch. f. exp. path. u. pharm., 1930, clii, 68.
(23) Gudjonsson, S. V. Journ. Physiol., 1932, lxxiv, 73.
(24) Hesse, R. Zeitschr. Zool., 1928, cxxxii, 314.
(25) Benedict, F. G. and J. F. Shell. Pflüger's Arch., 1902, xc, 33.
(26) Benedict, F. G. and E. G. Ritzman. Carnegie Inst. Wash., 1923, pub. *324*, 180.
(27) Benedict, F. G. and E. G. Ritzman. Carnegie Inst. Wash., 1927, pub. *377*, 142.
(28) Benedict, F. G. Carnegie Inst. Wash., 1915, pub. *203*.
(29) Johansson, J. E. Skand. Arch. Physiol., 1898, viii, 85.
(30) Lindhard. Meddelelser om Grønland. 1917, 44.
(31) Kohlschütter et al. cit. Luciani. Human physiology, 1917, iv, 461.
(32) Endres, G. and W. Frey. Ztsch. f. Biol., 1930, xc, 70.
(33) Hári, P. Biochem. Zeitschr., 1924, cxlix, 447.
(34) Givler, R. C. The ethics of Hercules. New York, Knopp, 1924.
(35) Benedict, F. G. and T. M. Carpenter. U. S. Dept. Agric. Off. Exp. Sta. Bull. 208, 1909.
(36) Becker, F. C. and O. Olsen. Skand. Arch. Physiol., 1914, xxxi, 81.
(37) Benedict, F. G. and C. G. Benedict. Proc. Nat. Acad. Sci., 1930, xvi, 438.
(37a) Rosenblum, D. E. Arb. Physiol., 1932, vi, 214.
(38) Gibson. Amer. Journ. Med. Sci., 1905, cxxix, 1048.
(39) Osborne, W. A. Journ. Physiol., 1908, xxxvi, proc. xxxix.
(40) Völker, H. Pflüger's Arch., 1926, ccxv, 43.
(41) Bunning, E., K. Stern and R. Stoppel. Planta (Berlin) 1930, xi, 67.
(42) Llewellyn, L. T. Nature, 1932, cxxix, 868.
(43) Marshall, F. H. A. Nature, 1932, cxxix, 344.
(44) Ramanathan, O. Nature, 1932, cxxx, 169.
(45) Dorno, C. cit. Dhar. The chemical action of light. London (Blackie) 1931.
(46) Purdy, C., A. F. Johnson and C. Sheard. Science, 1931, lxxiii, 46.
(47) Gessler, H. Pflüger's Arch., 1925, ccvii, 390.

(48) Bissonette, T. H. Science, 1932, lxxv, 18.
(49) Gessler, H. Pflüger's Arch., 1925, ccvii, 370.
(50) Wachholder, K. and F. Nothmann. Pflüger's Arch., 1932, ccxxix, 120.
(50a) Ogle, C. and Mills, C. A. Amer. Journ. Physiol., 1933, ciii, 606.
(50b) Giaja, J. Ann. de Physiol., 1932, viii, 535.
(51) Maignon, F. and J. Guilhon. Compt. Rend. Acad. Sci. Paris, 1931, cxcii, 1410.
(52) Benedict, F. G. and E. G. Ritzman. Carnegie Inst. Wash. Pub. *324*, 1923, 290.
(53) Tereg, J. In Ellenberger and Scheunert's Lehrb. d. Vergl. Physiol. d. Haussäugetiere. Berlin (Parey), 1910, p. 448.
(54) Marshall, F. H. A. Physiology of farm animals. Cambridge Univ. Press, 1920, p. 91.
(54a) Dill, D. B., et al. Amer. Journ. Physiol., 1933, civ, 127.
(55) Pembrey, M. S. Journ. Physiol., 1894, xvii, proc. xxvii, 1895, iii.
(56) Ginglinger, A. and C. Kayser. Ann. de Physiol., 1929, v, 710.
(57) Nicloux, M. Compt. Rend. Soc. Biol., Paris, 1931, cviii, 14, 17, 21.
(58) Giaja, J. and B. Males. Ann. de Physiol., 1928, iv, 875.
(59) Anderson, R. J. and G. Lusk. Journ. Biol. Chem., 1917, xxxii, 427.
(60) Benedict, F. G. and H. Murschhauser. Carnegie Inst. Washington, 1915, pub. *231*, 93.
(61) Deighton, T. Journ. Agric. Sci.,1929, xix, 140.
(62) Stigler, R. Arch. exp. Path. u. Pharm., 1930, clv, 257.
(63) Bazett, H. C. and B. McGlone. Amer. Journ. Physiol., 1926, lxxvi, 224; 1927, lxxxii, 415.
(64) Lomholt, S. Strahlentherap., 1930, xxxv, 324.
(65) Lees, Milner et al. In Smithsonian Physical Tables. Tab. 324. Washington, 1920.
(66) Barr, D. P. and E. F. DuBois. Arch. int. Med., 1918, xxi, 627.
(67) Ellis, N. R. et al. Journ. Biol. Chem., 1926, lxix, 219; 1931, xcii, 385 (others cited).
(68) Cathcart, E. P. and D. P. Cuthbertson. Journ. Physiol., 1931, lxxii, 349.
(69) van Dusen, M. S. In Internal. Crit. Tables, 1929, v, 228.
(70) Zummo, C. Bull. Soc. Ital. Biol. Sper., 1930, v, 450. Also Arch. di Fisiol., 1932, xxxi, 347.
(71) Cotte, J. Compt. Rend. Soc. de Biol. Marseille, 1931, cvii, 1131.
(72) Swift, R. W. Amer. Journ. Physiol., 1931, xcvii, 565.
(73) Shaw, L. A. and A. C. Messer. Amer. Journ. Physiol., 1931, xcviii, 93 (others cited).
(74) Kleiber, M. Hilgardia, 1932, vi, 315.
(75) Deighton, T. Journ. Agric. Sci., 1932, xxii, 418.
(76) Bauer, E. Biol. Zentralbl., 1931, li, 74.
(77) Berti, A. Arch. ital. de Biol., 1910, liv, 126 (cit).
(77a) Pickering, G. W. Heart, 1932, xvi, 115.
(78) Kirk, E. Skand. Arch. f. Physiol., 1931, lxi, 71.
(79) Cobet, R. T. et al. Zeitschr. f. exp. med., 1930, lxx, 739.
(80) Benedict, F. G. et al. Proc. Nat. Acad. Sci., 1932, xviii, 209.

(81) Halliburton, W. D. Text book of physiology. London (Murray) 1911.
(82) Mark, R. E. Deutsch. Arch. klin. med., 1928, clxii (cit).
(83) Wieland, H. Inaug. Diss. Wurzburg, 1929 (cit).
(84) de Bruin, M. Nederl. Tijdschr. Geneesk., 1931, ii, 4418 (cit).
(85) McGlone, B. and H. C. Bazett. Amer. Journ. Physiol., 1927, lxxxii, 452.
(86) Giaja, A. Compt. Rend. Soc. Biol. Paris, 1929, c, 1225; also Ann. de Physiol., 1931, vii, 13.
(87) Benedict, F. G. and C. G. Benedict. Proc. Nat. Acad. Sci., 1927, xiii, 364.
(88) Kriss, M. Journ. Agric. Res., 1930, xl, 271.
(89) Forbes, E. B. Proc. Am. Soc. Anim. Prod., 1925–6.
(90) Ritzman, E. G. and F. G. Benedict. N. H. Agric. Exp. Sta. Tech. Bull. 45, 1931.
(91) Krogh, A. Anatomie u. Physiologie der Capillaren. Berlin, 1924.
(92) Barcroft, J. Veter. Journ., 1931, lxxxvii, 466, 504.
(93) Cobet, R. and T. v. Haebler. Zeitsch. Klin. Med., 1929, cxii, 134.
(94) Rice, C. W. In Internat. Crit. Tables, 1929, v, 234.
(95) Griffiths, E. and A. H. Davies. Dept. Sci., Ind. Res. Food Invest. Bd. Sp. Rpt. No. 9 London, 1922.
(96) Aldrich, L. B. Smithsonian Misc. Coll., 1928, lxxxi, 6; 1932, lxxxv, 11.
(96a) Büttner, K. Klin. Woch., 1932, ii, 1508.
(97) Benedict, F. G. and H. S. Parmenter. Amer. Journ. Physiol., 1929, lxxxvii, 633.
(97a) Strauss, W. and S. Schwarz. Z. Hyg., 1932, cxiv, 42.
(98) Houghten, F. C. et al. Amer. Journ. Physiol., 1929, lxxxviii, 386.
(99) Benedict, F. G. and E. E. Croft. Proc. Nat. Acad. Sci., 1926, xi, 585.
(100) Benedict, F. G. and C. G. Benedict. Proc. Nat. Acad. Sci., 1927, xiii, 364.
(100a) Forbes, E. B., et al. Journ. Nut., 1932, v, 387.
(101) Waller, A. D. Proc. Roy. Soc., 1920, B. xci, 17.
(102) Stewart, G. N. Studies from the Physiol. Lab. of Owen's Coll. Manchester, 1891, i, 101.
(103) Cobet, R. and F. Bramigk. Deutsch. Arch. f. klin. med., 1924, cxliv, 45.
(104) Martin, C. J. Croonian Lectures, 1930 (pubd. Lancet 27, ix, 30).
(105) Keller, P. Strahlentherap., 1930, xxxv, 353.
(106) Cobet, R. and H. Kionka. Zeitschr. Physik. Therap., 1929, xxxvi, 160.
(107) Phillip, H. Zeitschr. Physik. Therap., 1930, xxxviii, 177.
(108) Angus, T. C. and H. J. Taylor. Proc. Roy. Soc., 1931, B. cix, 309.
(109) Meyer, H. and H. Kirchoff. Deutsch. med. Wochenschr., 1932, i, 41.
(110) Plotnikow, J. and R. Mibayashi. Strahlentherap., 1931, xl, 546.
(111) Wiggers, C. J. and O. Orias. Amer. Journ. Physiol., 1932, xc, 614.
(112) Dufton, A. F. Phil. Mag., 1930, ser. VII, ix, 858.
(113) Marsh, F. Journ. Physiol., 1930, lxx, xxxviii.
(114) Aschkinass, E. Ann. d. Phys. u. Chem., 1895, lv, 401.
(115) Dreisch, T. Zeitschr. f. Physik., 1924, xxx, 200.
(116) Hill (cit. Laurens). Physiol. Revs., 1928, viii, 1.
(117) Stigler, R. Wien. Med. Wochenschr., 1929, No. 31.
(118) Bachem, A. and C. I. Reed. Amer. Journ. Physiol., 1931, xcvii, 86.

(119) Lucca, A., and Reviglio, G. M. L'Actinoterapia, 1929, viii, 153.
(120) Laurens, H. Physical. Revs., 1928, viii, 1.
(121) Gigon, A. and M. Noverraz. Zeitschr. f.d. ges. exp. med., 1930, lxxi, 525 (cit).
(122) Franz, H. Biol. Zentralbl., 1930, l, 158.
(123) Palit, C. C. and N. R. Dhar. Journ. Physical. Chem., 1928, xxxii, 1263; 1930, xxxiv, 993.
(124) Küstner, H. Zentralbl. Gynäk., 1931, 2986 (cit).
(125) Reviglio, G. M. L'Actinoterapia, 1928, vii, no. 4.
(126) Schear, E. W. E. Amer. Journ. Physiol., 1932, xcix, 535.
(127) Nasset, E. S. Amer. Journ. Physiol., 1932, ci, 194.
(128) Hodgson, R. E. et al. Journ. Agric. Res., 1932, xliv, 357.
(129) Goldhammer. Dispersion u. absorption des lichtes. Leipzig (Teubner), 1913 (cit.).
(130) Egerton, G. F. In Internat. Crit. Tables, 1929, v, 53.
(131) Soderstrom, G. F. and E. F. DuBois. Arch. Int. Med., 1917, xix, 931.
(132) Newburgh, L. H. Amer. Journ. Physiol., 1931, xcvii, 548.
(133) Wiley, F. H. and L. H. Newburgh. Journ. Clin. Invest., 1931, x, 689, 723.
(134) Jores, A. Zeitschr. f. exp. med., 1931, lxxvii, 734.
(135) Levine, S. Z. and E. Marples. Amer. Journ. Dis. Child., 1930, xl, 269.
(136) Manchester, R. C. et al. Journ. Nut., 1931, iv, 39.
(137) Lazlo, D. and A. Schurmeyer. Zeitschr. klin. med., 1931, cxvi, 22.
(138) Benedict, F. G. and H. F. Root. Arch. Int. Med., 1926, xxxviii, 1.
(139) Kuno, Y. Journ. Orient. Med., 1931, xv, 99.
(140) Houghten, F. C. and C. P. Yagloglou. Trans. Amer. Soc. Htg. and Vent. Engrs., 1924, xxix, 163, 361; xxx, 193.
(141) Viale, G. Arch. per le sci. med., 1920, xliii, 40.
(142) Lazlo, D. Verh. Deutsch. Ges. inn. med., 1930, xlii, 93.
(143) Whitehouse, A. G. R. Proc. Roy. Soc., 1931, B. cviii, 326.
(144) May, E. Zeitschr. klin. med., 1931, cxvi, 14.
(145) Vasti, A. Amer. Journ. Physiol., 1932, cii, 59.
(146) Shaw, L. A. and A. C. Messer. Amer. Journ. Physiol., 1930, xcv, 13.
(147) Murschhauser, H. and H. Hidding. Biochem. Zeitschr., 1912, xlii, 357.

RADIATION-GENETICS

FRANK BLAIR HANSON

Washington University, St. Louis, Mo.

The cause, or causes, of heritable variations in organisms has been a fundamental biological problem since the time of Darwin. It became an imperative challenge with the great development of the science of Genetics in this country.

Heritable variations, or mutations, are facts of nature, else there could have been no evolution. Modern experimental methods over a long period of years failed to induce or duplicate the known mutations of spontaneous origin. Yet work on a large scale and covering most of the species suitable for laboratory experimentation was not lacking. The literature of the failures to modify the germ plasm is too extensive for review here and unnecessary, except for that of one species of the fruit fly (*Drosophila melanogaster*) upon which the major part of this paper is based and which was the material that finally yielded the secret of artificially produced mutations by means of x-rays in 1927 (Muller, 1927, 1928).

Back of Muller's success with the x-ray lies his own work of some eight years of experimentation with the effect of temperature on *Drosophila*. Negative results were the reward of other workers also. Payne (1911) bred sixty-nine generations of flies in a dark room. At the end of this period the flies were tested for their reaction to light, but showed no significant differences from the controls. Duncan (1915) was unable to increase the mutation frequency by crossing races from widely separated localities. Morgan (1910, 1911, 1914) used "wide ranges of temperatures, salts, sugars, acids, alkalis, without resulting mutations." Some mutations did appear (1911) after treatment with radium, but failed to reappear upon repetition of the experiment, also including the use of x-rays. Loeb and Bancroft (1911) likewise reported mutations in flies after radium treatment, but Morgan apparently was able to show that these mutant types were already present in the stock. Guyénot (1914) got negative results with high temperature, radium, x-rays and ultraviolet. Since all laboratory cultures of flies are etherized once or several times for purposes of handling and examination, Morgan tested the

theory that ether might be the cause of mutations by treating flies with a near lethal dose of ether. No mutants were found among the 31,168 offspring examined. Mann (1923) treated flies with high and low temperature, and a number of chemicals including, arsenic, methyl alcohol, ethyl alcohol, quinine, morphine, copper sulphate, lead acetate, etc. The number of mutants in the treated flies was actually less than in the controls. Many experiments on temperature effects in flies performed by Northrop (1920), Plough (1917), Krafka (1920), Plough and Strauss (1923) were all negative.

Nevertheless, as it became increasingly clear that gene mutations probably form the chief materials of organic evolution, some measure of control over the mutation process was essential for the solution of fundamental problems.

In 1927 Muller announced that he had obtained mutations, both visible and lethal, in *Drosophila* following treatment with x-rays. The next year (1928) he also announced success with the effects of temperature, as has also Plough (1932). Thus, after many attempts by numerous investigators over a period of years, we now have two external agents which act on genes to produce mutations. X-rays and radium were used as early as 1911 to induce mutations in flies, but without success. For one thing, Muller succeeded where earlier workers failed because during the intervening years many new methods of breeding and the origination of specially "made-up" stocks had been developed to a high degree. Muller, himself, was responsible for many of these advances in breeding stocks.

Muller's work was confirmed almost immediately by Weinstein (1928), and others, and extended to radium by Hanson and Stadler. Soon a host of workers in America and Europe were making use of the new methods and in the ensuing five years they have been applied to a large number of species, both plant and animal.

It is not the purpose of this paper to give a complete review of Radiation-Genetics, but rather to consider in some detail special problems that are of considerable biological significance and that can be illustrated largely by work done with *Drosophila* flies, introducing work with other animal or plant materials only as they throw light on the subjects chosen for discussion. To this end the important work of such investigators as Goodspeed (1932, and earlier papers), Blakeslee and his collaborators, Bagg and Little (1924), Whiting (1929, 1932), and many other contributors, could not be brought within the space limits of this review.

There are approximately two hundred papers on the subject, all of them dating since 1927.

ARE "NATURAL MUTATIONS" DUE TO "NATURAL RADIATION?" Muller (1927) in his now famous paper before the Fifth International Congress on Genetics at Berlin first raised the question that heads this section. Since then Olson and Lewis (1928), Haldane (1929), Joly (1929), Babcock and Collins (1929, 1929), Hanson and Heys (1930), Broadfoot (1932), and others, have suggesed that possible radiations from the earth, or even cosmic rays may have played an important rôle in the evolution of species by furnishing heritable variations for the action of natural selection.

These suggestions have borne fruit in two experiments quite independently carried out by Babcock and Collins, and by Hanson and Heys, in an effort to measure any differential in the mutation rate between two localities showing a known difference in the amount of ionizing radiation present.

Using an electroscope Babcock and Collins discovered a location in a street-car tunnel in San Francisco where the natural ionizing radiation was fully twice as great as the radiation in their laboratory in Berkeley. Accordingly their experiment was designed to compare the rates of occurrence of sex-linked lethal mutations in *Drosophila* in the street-car tunnel and in the laboratory. The lethal mutation rate in Berkeley was 0.26 per cent, and in the tunnel 0.52 per cent, the difference between the two rates being 2.5 times the probable error. Upon a reanalysis of the data showing the actual experimental variation in rate in the several sub-groups in each of the two series it was found that the difference between the average rates for the two localities was increased. This difference was 0.275 ± 0.086.

Hanson and Heys, in accepting the implied challenge to experiment contained in Olson and Lewis' paper, considered themselves suitably located for such an undertaking, i.e., near the Ozark caves and lead mines of Missouri. But a long search with the electroscope in the numerous caves and lead mines of this region failed to reveal a location with a sufficient increase of ionizing radiation over that of the laboratory, or the middle of a Missouri cornfield, to justify breeding experiments.

Operations were transferred to Colorado. In the East Paradox Valley of Western Colorado in an abandoned carnotite mine the air was strongly ionized. In addition to the electroscope readings a rough attempt was made to compare the amount of natural ionization in this mine with that of 1 mgm. of radium. Radiation in the mine was found to be 0.39 times as intense as that from 1 mgm. of radium when the rays were passed through a 0.156 inch lead filter.

Male flies were exposed in this mine for 140 hours and then returned to St. Louis for the breeding tests. The well known C1B method for detecting lethal mutations was employed.

The frequency rate of the test cultures was 0.245 ± 0.062 per cent. The difference between tests and controls is 0.169 ± 0.081, a difference of 2.09 times its probable error.

These two experiments, one in California and one in Colorado, while falling short of being statistically significant, nevertheless are consistent in that both give an actually higher rate of mutation in flies exposed to natural radiation than in the controls. They seem to strengthen definitely the plausibility of the suggestions quoted above to the effect that natural radiation may be responsible for the mutations which are the grist of the natural selection mill with the resulting evolution of new forms.

However, Muller and Mott-Smith (1930) by ingenious mathematical computations have shown that the natural mutation frequency is at least thirteen hundred times as high as it would be if it were caused solely by the radiation which flies receive from their outer environment. Similar measurements by these two authors seem to eliminate radiations from the fly food and from the cells of the flies themselves as effective causes of the natural frequency of mutations.

We are, accordingly, left in this anomalous situation: direct experimentation indicates that in a locality (street-car tunnel, Carnotite mine) where the natural ionizing radiation is approximately doubled there is an increase in mutation frequency, but on the basis of Muller and Mott-Smith's calculations this is wholly inadequate to account for the majority of mutations occurring in nature and therefore for organic evolution. So most mutations must come about as a result of other causes. These causes are at present unknown. At the same time it remains true that gene mutations resulting from radium and x-rays are indistinguishable from those occurring naturally; therefore the geneticist can still use this method, the only one known at present, to study the problems of the mechanism of mutation and the physical basis of heredity.

Cosmic rays. Great interest attaches, at the present time, to the so-called cosmic rays. Since as shown above there is an increasing proportion of mutations produced as we pass from ultra-violet through the x-ray part of the spectrum and on to the gamma rays, i.e., from longer to shorter wave lengths, it is not surprising that geneticists and others interested in the biological effects of radiation should speculate

concerning the possible biological effects of these shortest of all known rays—the cosmic rays.

Joly (1929) and Joly and Dixon (1929) suggest that changes in intensity of cosmic radiations may have influenced the incidence of malignant disease and think that experiments at high altitudes to test the progress of malignancies should be tried. According to Joly there are no sure grounds for believing that cosmic rays are uniformly distributed in place or time and he makes the suggestion that the recent world-wide increase in cancer may be due to a decrease or low point in a cosmic ray cycle. Of greater interest to the geneticist is his other suggestion that cosmic rays may have been a factor in the production of variations in animals and plants by direct action on the germ plasm. Orton and Burfield (1931) offer the suggestion that these rays entering the earth from space may be a normal requirement of at least certain organisms.

No experiments, so far as known to the writer, have been attempted along these lines. The technical difficulties and the expense involved, together with the uncertainties still existing regarding the true nature and source of cosmic rays, so far have deterred biologists from entering this field. Of course if the results of Muller and Mott-Smith reported above are substantially correct there is no possibility that the cosmic radiation could play a significant rôle as a cause of mutations, since the ionization produced by it is considerably less than that produced over the land by the radiation from terrestrial sources.

PROGRESSIVE MUTATIONS. Critics of the mutation theory of evolution, implying that mutations are the "building blocks" of evolution, point out that most mutations are deleterious in their effects and hence could not have been the underlying cause of progressive evolution. It is true not only of the x-ray mutations but also of the natural mutations that they are more often degressive than progressive changes.

Muller (1927, 1930) has discussed this criticism in several of his papers and points out that this is one of the most vital and immediate questions confronting x-ray genetics, for upon the answer to it may depend in turn the answers to fundamental questions concerning the mechanism of evolution, the nature of the gene, and the means of bringing about artificial evolution.

It must be admitted at the outset (Muller, 1930; Patterson and Muller, 1930) that there are two main facts which indicate that radiations may not be constructive in their genetic action: 1, they do often cause "destructive" changes, and 2, they cause breakage of chromosomes, accompanied by the loss of fragments or their reattachment at a differ-

ent point in the chromatin (Muller, 1927; Weinstein, 1928; Muller and Painter, 1929; Painter and Muller, 1929).

That the above should be true is both an *a priori* expectation and a generally observed fact, for any interference with the delicate gene mechanism is more apt to result in an "upset" than in a progressive change (Muller, 1923).

But are mutations always retrogressive changes? Are the x-ray or radium rays, as Altenburg has inquired, merely punching holes in the chromosome, i.e., causing losses of chromatin or inactivations of genes?

The answer is in the negative. Clear-cut *reverse* mutations have been obtained in corn, *Drosophila*, *Portulaca* and elsewhere. It is evident (Muller, 1929) that if x-rays induce a change of a gene designated as large *A* to a mutant gene designated small *a*, and small *a* represents a destructive process or loss, then the opposite or reverse mutation from small *a* to large *A* must represent a constructive process or gain.

A case of a spontaneous dominant mutation, the ragged character, in maize has been described by Brink and Senn (1931). Lindstrom (1925) records another dominant in maize (Teopod). Horlacher and Killough (1932) report a mutation in cotton, after raying, from a recessive virescent yellow to a dominant normal green chlorophyll condition. Karper (1932) records a dominant mutation in sorghum that has been appearing regularly for the past fifteen years.

Experimental evidence now to be presented actually realizes the accomplishments of the fabled man who jumped into a bramble-bush and scratched out both his eyes, only to jump into another bush and scratch them in again.

Muller (1930) gives convincing evidence that changes other than losses can be produced by radiation. His best cases are perhaps those of seven reverse mutations in which the gene for forked bristles in *Drosophila* reverted to the dominant normal bristles and a true breeding stock of the reversed gene was established. In this experiment "contamination" clearly was ruled out as the chance of obtaining all seven independent reverse mutations in the treated and none in the control group (as was the fact) would have been 1 in 128 if the irradiation had not been effective in producing these mutations. The chromosome carrying the gene for forked bristles also had the gene for tinged eye which was used as a marker to further insure against contamination.

Muller (1930) then undertook experiments with the stock of tinged, non-forked flies that had been derived by reverse mutation from forked to determine whether this stock could be caused to mutate again to

forked. The results were two inheritable mutations to forked and two "fractionals" in a count of 58,817 progeny of treated flies. None was found in 34,798 controls. In some previous experiments Muller had obtained three other mutations to or toward forked. This total of five heritable and two fractional mutations to forked in radiated material proves beyond question that irradiation was the cause of the mutations in this direction also. In these experiments then we have a complete cycle (forked⇄non-forked) produced by raying. Gene mutations at the locus forked, and probably elsewhere, are of the nature of a two-way reaction system.

Simultaneously with the above described work of Muller, papers on the same subject appeared by Timofeeff-Ressovsky (1929, 1930). Timofeeff-Ressovsky rayed eggs and larvae of white and eosin-eyed flies in an effort to induce changes in eye-color in opposite directions. Two cases of reversion to red and one to an intermediate color were found. Later Timofeeff-Ressovsky irradiated adult males having various allelomorphs of white and obtained 13 reversals to the normal. In most of these cases it was possible by suitable breeding tests to prove that the change was genetic. In addition Timofeeff-Ressovsky was able to get at four different loci in the sex chromosome mutations in the opposite direction, i.e., from abnormal to normal.

Hanson (1928) x-rayed male *Drosophila* having in the X-chromosome the gene which produced the bar eye. These irradiated males (T4 and T8 doses) were mated to virgin double-X yellow females. These females are peculiar in that the two X-chromosomes are attached at the right hand end and go together into the same gamete, which is equivalent to 100 per cent non-disjunction. The double-X yellow females also carry a male Y-chromosome. In such a cross the sons get their X-chromosome from their fathers and their Y-chromosome from their mothers, a reversal of the usual procedure in this species. The advantage of this method (used also by Muller and Timofeeff-Ressovsky) is that mutations occurring in the X-chromosome of the treated fathers show up in the first generation sons, whether recessive or dominant, as they are not covered by normal allelomorphs in the Y-chromosome. In this experiment there were four cases of reversion to full eye, and these bred true to full eye. Two were in the T-4 treatment group and two in the T-8 group; a ratio of one reversion in 433 males in the T-8, one in 1,898 in the T-4. These four reversions from bar to full eye are of considerable interest. Zeleny (1921) has studied the bar gene very extensively. He found that bar eye reverts to round eye about once in

1600 times; and also discovered an allelomorph of bar, which he called ultra-bar, the reversion rate of which is approximately the same. Zeleny considered the bar-eye character to be due to a gene mutation not different from other gene mutations in *Drosophila*. Upon evidence based on the sex-ratio Zeleny concluded that mutations to full eye may occur in the male germ tract as well as in the female. Sturtevant (1925), however, reports extensive experiments which seem to show that reversions at the bar locus are due, not to gene changes at all, but rather to unequal crossing-over between the two X-chromosomes of the female. Hence, according to Sturtevant, no reversion in the male is possible, since no crossing-over occurs in the male.

However, that reversions to round eye are possible in the male seems evident by the four reversions in males secured by Hanson, and also that reversions in the female are possible without crossing-over is indicated by unpublished work of Muller who obtained two reversions to round eyes in females in cases where there was no crossing-over near the bar locus, one in control material and one in lightly x-rayed flies.

In the tiny fourth pair of chromosomes of *Drosophila* there is a gene mutation reducing the size of the eyes. This is a recessive gene and could be termed a loss. Recently, however, an "eyeless" (reduced eye) mutation, similar in appearance to the old one, has been obtained by raying, but this new mutation, unlike the old "eyeless" is a dominant, and therefore could not be of the nature of loss, since the real loss, old "eyeless," gives no dominant effect (Muller, 1928).

Similarly in the case of the mutant character called "Scute," in which the scutellar bristles are absent, return mutations to the normal condition have been observed to occur both spontaneously and after raying. Muller (1929, 1930) concludes on the basis of the above findings that mutations arising as a result of x-raying are not always or merely destructive changes and losses. If some are to be termed losses, others as certainly must be of the nature of gains. Or preferably, since mutations are of the nature of chemical reactions, these are neither losses nor gains, but rather substitutions and rearrangements of genetic materials. But the fact remains that "progressive" mutations can be produced by radiation and they also occur spontaneously. Hence evolution by the natural selection of mutations which happen to be adaptive in character is at least a strong probability.

DIFFERENTIAL SUSCEPTIBILITY OF EARLY AND LATE GERM CELLS TO IRRADIATION. In Muller's earliest experiments the flies hatched from eggs laid seven to twelve days after raying of the sperm which fertilized

them showed the same percentage of mutations as those hatching from eggs fertilized by sperm during the first six days following treatment.

Hanson and Heys (1929) and Harris (1929) independently raised the question as to the ultimate duration of the effect of irradiation upon mature sperm and as to whether there is a differential effect of the same dose upon the various stages of spermatogenesis.

Hanson and Heys (1929) rayed males and mated these males to C1B females, thus using a method which reveals percentage of lethal mutations. Transfers of these parents were made to new food bottles at

TABLE 1

BROODS	AGE OF GERM CELLS IN DAYS AFTER IRRADIATION	PER CENT MUTATIONS
I	1–7	13.00
II	7–14	14.20
III	14–21	1.01
IV	21–28	4.30
V	28–35	0.00

TABLE 2

BROODS	AGE OF GERM CELLS IN DAYS AFTER IRRADIATION	PER CENT MUTATIONS
I	1–4	8.6
II	4–8	9.7
III	8–12	7.3
IV	12–16	1.7
V	16–20	0.6
VI	20–24	0.8
Controls		0.2

seven day intervals for a period of thirty-five days. There were, therefore, five broods of young and the mutation percentage for each brood is given in table 1.

These results show that for fourteen days after raying there is no appreciable change in the mutation rate. In the next period a sharp drop occurs; there is a slight rise in the fourth brood and in the fifth the zero point is reached.

Harris (1929) performed essentially the same experiment with the exception that the interval was four days instead of seven. His table here given shows the same sharp decrease in mutation rate but after the twelfth day instead of the fourteenth as in Hanson's experiment (table 2).

The most reasonable explanation of the tables as interpreted by Hanson and by Harris seems to be that mature sperm present in the fly at the time of raying give an approximately constant rate of mutation for twelve to fourteen days after treatment. By the end of this period new sperm are matured which during the raying some two weeks previous were in the earlier stages of spermatogenesis. There is, therefore, a differential effect of the rays upon immature and mature male germ cells. This is, according to Hanson, in line with expectations if the mutational effects are due to a bombardment of electron bullets. In the head of the mature sperm the chromosomes are packed solidly in small compass while in the immature stages the nuclei are large and the chromatin material is much scattered allowing most of the electrons to pass through the chromatin-empty spaces of the cell. We should expect therefore that many more hits would be registered on the mature germ cells and that when these are used up the new sperm should show a much lower mutation rate, which is actually the case. Harris (1929) has suggested an alternative interpretation, namely, that immature germ cells in which zygote-lethal changes have been produced tend to be killed or to multiply much less rapidly than the genetically normal cells, a condition representing a kind of "germinal selection." This also would account for the sharp drop in the mutation rate twelve to fourteen days after raying.

X-RAYS AND SOMATIC MUTATIONS. Muller's discovery that by treating fruit flies with x-rays the germinal materials may be altered so as to give mutations that breed true through hundreds of successive generations has opened up also a number of other fruitful fields for investigation by similar methods.

One of these important fields, that of the physiology of development, has already yielded results through the work of Patterson (1928, 1929, 1930), Timofeeff-Ressovsky (1928, 1929) and Hance (1927). At the time of fertilization the zygote receives from the male and female gametes a certain definite double set of genes or hereditary determiners. By means of the longitudinal splitting of the chromosomes of the fertilized egg both cells of the two-celled stage receive the same kind of chromosomes and therefore the same gene complex as the original cell. By the same token all the cells in the body have the same gene determiners as the egg.

Now one of the most important problems in experimental embryology concerns the manner in which a specific gene behaves during the formation of a structure. If by some artificial means a gene in a somatic

cell could be made to mutate or could be destroyed, or eliminated from the cell, then a study of the descendant cells might throw some light on the problem of development. X-rays have proven to be the means of accomplishing both gene mutations and chromosome derangements in somatic cells as well as in germ cells.

Patterson used the sex-linked genes for eye-color in *Drosophila* as probably the most suitable materials for a study of this kind. *Drosophila* flies have the usual compound eye found in insects which is very large and made up of a series of definite units called ommatidia. A large number of multiple allelomorphs for eye-color in this species are well known and changes in color in one or a group of the ommatidia can be detected and counted. Patterson mostly used the plan of crossing flies carrying the dominant genes for red eyes (wild type) with flies having recessive allelomorphs.

The heterozygous offspring of such crosses were irradiated at various stages of development—some in the egg stages, others at timed intervals during larval and pupal life. Since the eye colors used are sex-linked all females are heterozygous, but the males having only one X-chromosome are either the dominant red or one of the recessive allelomorphs. It is of advantage to use heterozygous females because even with the great acceleration of mutations due to x-rays it would not often happen that the two dominant genes in a homozygous fly would be caused to mutate simultaneously. If, however, the females are heterozygous then a change in the dominant gene to one of the lighter colors could be observed in the affected eye facets in various combinations of the eye-color allelomorphs. Patterson irradiated 28,821 eggs and larvae,—and from these there developed 11,468 flies. The 6,084 females from treated cultures yielded 401 flies with a total of 429 modified ommatidial areas, while 5,384 males gave only thirty-nine individuals with forty-one such areas. It is apparent that the color change was produced about nine times more frequently in females than in males. No eye color change occurred among the 11,100 control flies.

Patterson presents evidence which seems to show that color changes in the males are due to gene mutations, while in the females in addition to a proportionate number of gene mutations there occur also chromosome breakages and translocations.

The size of the modified color areas has no relation to the length of treatment given, but is determined by the age at which the culture was rayed. When eggs or young larvae are treated, the mutant areas are large, but if treatment be given in mid- or late-larval stages, small areas

result. Treatment of the pupal stage is ineffective. This means that if a mutation occurs in early developmental stages the white area of the eye will be enlarged by cell divisions of the mutated cell. If the cell is in a late larval stage it will have few cell descendants and hence give rise to a small modified area. For example, if the mutation occurred in one of the first two cells of the eye rudiment, one-half of the eye will be white, but if the mutation is induced in one of the first four cells, approximately one-fourth of the eye will be white.

These results were confirmed by the work of Timofeeff (1928, 1929), and are considered further in the section on the rate of mutation and reverse mutations.

PHYSIOLOGICAL STATES AND THE INDUCED MUTATION RATE. Muller (1930), Stadler (1928) and Hanson and Heys (unpublished data) have investigated the influence of various internal and external factors acting in association with x-rays on the rate of mutations.

Muller tested the effect of temperature in one experiment and of starvation in another. In the former type of experiments two batches of flies of identical origin were heavily irradiated, but during treatment one was kept at a temperature of 34°C. and the other at 8°C. Twenty-two of the 67 F_1 cultures in the cold series, or 33 per cent, showed lethal mutations, and 32 of the 120 F_1 cultures in the warm series, or 27 per cent, showed such mutations. This is not a significant difference. Since these numbers were small the experiment was repeated on a large scale, the results being, however, the same. Stadler also obtained similar negative results in experiments in which barley was subjected to different temperatures while being x-rayed. Muller concludes that there is no great *rise* in the mutation rate with increasing temperature such as occurs in the case of an ordinary chemical reaction where an increase of 26°C. in temperature raises the rate of chemical reaction from six to sixteen times.

Muller also studied the effect of the metabolic rate on the mutation rate. It is known that anabolism and multiplication of oögonia and oöcytes are more active in well-fed females than in starved ones, and also in impregnated females than in virgins (Hanson and Ferris, 1929). Muller obtained negative results when comparing the number of lethals in fed and starved females, and in cases of rapid and slow germ cell formation. But his numbers were very small and the importance of the problem suggested to Hanson and Heys that a repetition on a large scale was indicated.

Therefore these last mentioned authors repeated the experiments

on starvation, rapid and slow germ-cell formation and added a third variable not previously studied—namely, the effect of complete ether anesthesia during irradiation on the number of lethal mutations produced. The effect of age is now under investigation.

a. Irradiation after starvation. There can be little doubt that tissues and cells differ in susceptibility and that the sensitivity of a given cell may change from time to time. The same intensity of radiation produces very dissimilar effects upon different kinds of cells and upon cells in different phases of activity or of their life histories. As early as 1906 Bergonié and Tribondeau concluded that sensitivity varied directly with reproductive capacity and inversely with the degree of differentiation of the cells. Curtis (1928) observed in Planaria that undifferentiated formative cells readily succumbed to x-rays, although the animal itself may continue to live for a long time.

Cells undergoing mitotic division are considered by most authors to be highly susceptible to radiation, although there is a wide divergence of opinion as to which is the critical mitotic phase.

Vitemberger (1928) concludes that susceptibility gradually increases as mitosis proceeds and then falls sharply at the telophase. Strangeways and Hopwood (1926), studying the effects of x-rays upon mitotic divisions in vitro, considered the brief period of organization just before division the high point of sensitivity, while Regaud in 1923 recorded two maxima during the process itself, the prophase and the anaphase.

Cytological studies and genetic experiments both give evidence that some particular part of a cell may be altered while the remainder suffers no perceptible damage. Jannson in 1927 observed profound modifications of the nuclei of cells after exposure such as fragmented and misshapen chromosomes, attachment of the division halves by chromatin bridges, etc. Although the continuance of development is impossible where injury is severe, Strangeways and Hopwood (1926) have found that when a cell is irradiated after the mitotic process has been initiated, the division continues in spite of injury. Canti and Donaldson (1926), also studying the effects of radiation on mitosis in vitro, found that even a brief exposure to the gamma rays of radium prevented cells which were about to divide from beginning the process. Unless, however, irradiation is long-continued or very intense, most cells suffer no visible injury but begin to divide again after a short recovery period, giving no evidence of permanent harm. In tissue culture work in which these phenomena have been observed to particular advantage, the temporary check in cell division is followed by a considerable in-

crease in the number of dividing cells (Regaud, Lacassagne and Jovin, 1925).

This return to activity after relatively slight exposure, and the fact that in tissues or cells under irradiation some part will respond more quickly than another, form the basis of the entire field of those genetic experiments in which lethal and visible mutations, translocations and gene rearrangement have been produced experimentally in an attempt to solve the problem of the causes of variability among organisms in nature.

That internal conditions do affect the frequency of mutations produced by irradiation is shown also by such observations as the higher rate of induction in mature sperm than in adult females and larval males (Muller, 1930). Hanson (1929) and Harris (1929) independently found that the rate of lethals from mature sperm was five or more times higher than from spermatogonia treated in the adult male. Muller (1930) found also a sex difference in the rate of mutation under identical conditions of treatment. It is now generally accepted that this differential behavior of the cells under irradiation is *not* due to any selective power of the rays, since all parts of the cell and all kinds of cells have about the same power of absorption. Whether the cell is affected or what part of it is altered seems to depend upon its physiological condition at the time of irradiation and on the consequent physical and chemical changes.

Cultures of wild type *Drosophila*, already having minimum food supply, were subjected to overcrowding by increase in the per-culture population. In these cultures both the pupae and the flies hatching from them are very small and show obvious signs of starvation. Male flies collected from such cultures were completely starved after hatching for a period of 8 hours and then were given one hour's exposure to 300 milligrams of radium (gamma radiation only). The alpha and beta rays were excluded by means of a 0.5 mm. platinum filter which at the same time allows the passage of 78 per cent of the gamma radiation. Secondary radiation was reduced to a minimum by filtration through a layer of Columbia paste, 1 cm. in thickness. The flies were enclosed for treatment in thin gelatin capsules. Following treatment these starved males were mated to well-fed C1B females of the stock.

The results obtained differ significantly in two respects from those observed with unstarved treated controls, namely, sterility of the parent and F_1 generations and lethal mutation rate. Mortality in the parent generation, based on the number which had died by the second day

after exposure, was high: 11.40 ± 0.9586 per cent in a total of 500 P_1 cultures, as compared with no mortality in the controls. This high rate of post-irradiation mortality is indicative of the severity of the starvation, 11.40 per cent being unable to survive the combined effects of inanition and irradiation. The percentages of sterility in both generations and the rate of lethal mutation were significantly lower than in the controls. As in the experiments dealing with equivalent dosages (Hanson and Heys, 1932) these sterility values are absolute, representing complete sterility. Sterility tests were carried out in the same manner as before. It is interesting to note that here also as in the proportionality experiments the amount of sterility occurring in the second generation is approximately one-half that in the treated generation.

First generation bar-eyed females of the cross C1B by wild were selected for treatment. Such females, having one X-chromosome carrying the C1B factors and one X-chromosome carrying the wild-type are heterozygous for the lethal character and when mated to the wild type give one-half the usual number of males in the F_2 generation. If a new lethal mutation were induced in the chromosome bearing the wild-type characteristics, the other half of the sons would inherit it and also fail to appear. Preliminary experiments demonstrated the occurrence of an induced lethal in this X-chromosome.

These F_1 females were subjected to starvation in exactly the same way as the males previously described and exposed to the same dosage, 300 mgm. for 1 hour. The results confirm those obtained earlier with wild-type males where the lethal is carried by the F_1 female and detected in the second generation. Here again, both the sterility and the mutation percentages are significantly lower than those in unstarved treated controls.

Concerning the comparative effects on the two sexes, the mortality, sterility and mutation values are consistently less when females are treated, although not significantly so. Muller (1930) has found some indication of sex differences, the "rate of induction in mature sperm" being "higher than that in adult females."

Evidence from a number of sources shows that the cells of starved tissues undergo division less and less frequently as starvation continues and then remain in a resting condition until degenerative changes set in. This mitotic inactivity accompanying inanition has been observed particularly in work on transplantation and grafts, and also in tissue culture work, where normal tissue culture cells are deprived of nutritive fluids. Since cells initiating the division cycle occur very infrequently

and those in the resting phase are less susceptible to the effects of radiation, it seems logical to suppose that the marked decrease in radiation effectiveness observed in the starved tissues in these experiments might be attributed to a decrease in mitotic activity.

b. Irradiation during complete ether anesthesia. *Drosophila* flies were here irradiated under identical conditions except that in one batch they were under complete ether anesthesia while in the other the flies were awake and active. The anesthetized flies gave a lethal mutation rate of 13.99 per cent for the males and 13.02 per cent for the females as compared with 9.74 per cent for the males and 8.48 per cent for the females in the non-anesthetized flies. For the males this is a difference of 4.52 times the probable error and for the females a difference of 6.33 times the probable error, both clearly significant differences. The differences in sterility were equally significant in favor of the flies rayed during anesthesia.

It is evident, then, that radiation during starvation gives a lower mutation rate and radiation during anesthetization a higher mutation rate than in the corresponding controls. As noted above, the authors suggested that starved tissues divide less frequently and finally come to a resting condition, which is the stage where radiation is least effective. In the case of anesthesia it is conceivable that ether absorbed by the germ cells may act as a sensitizer in combination with radium-radiation and may have a catalytic action for the type of chemical transformation which results in the production of lethal mutations. Changes in protoplasmic viscosity may be brought about by irradiation which might have considerable influence upon the response of the cell to absorbed chemical substances. Haendly (1918), studying changes in irradiated carcinoma cells, observed marked alterations in the degree of hydration and of viscosity, increase or decrease in nuclear size, aggregation or disintegration of chromatin, etc. That sol⇄gel changes occur in the colloidal constituents of nerve cells during anesthesia is generally accepted. Possibly it is not running too far afield to suppose that similar changes in germ cells might sensitize them to radium radiation.

c. Irradiation during rapid vs. slow germ cell formation. Observations reported from time to time by numerous investigators show that rapidly dividing cells are particularly susceptible to the effects of irradiation of various kinds (Bergonie and Tribondeau, 1906; Regaud, 1922, 1923; Strangeways and Hopwood, 1926; Vintemberger, 1928; Hertwig, 1911; Mottram, 1913; Lazarus-Barlow and Beckton, 1913; Regaud, Lacas-

sagne and Jovin, 1925; Stadler, 1928a, 1928b; and Contamin, 1909, 1910).

Hanson and Ferris (1929) have shown that the egg-laying capacity of mated *Drosophila* females is much greater than that of virgins. The peak of egg production comes on the fourth day after hatching and it may be supposed that oögonial and oöcyte divisions must be going on at the most rapid rate just previous to the peak of egg production. Hanson and Heys (unpublished data) have compared the mutation rates after radium radiation in impregnated females in which oögonial division is going on at a rapid rate with the mutation rates in virgins (unmated until after raying) where oögonial activity is at a relatively low ebb. The offspring of the impregnated females showed 14.44 per cent lethal mutations as compared with 8.54 per cent in the virgins, a clearly significant difference. These results are interpreted tentatively to indicate a greater sensitivity to radiation on the part of the rapidly dividing germ cells of mated females as compared with diminished sensitivity of the sluggishly dividing germ cells in unmated females.

NATURAL AND INDUCED MUTATION RATES. The basic problems of evolution today relate to the nature, causation and modes of transmission of heritable variations. Geneticists are chiefly concerned with rare, sudden changes in those character-determining particles called genes. For it is the genetic doctrine that mutations occurring in the genes furnish the stuff from which evolution proceeds.

Mutations are, apparently, as universal as species are; but under natural conditions they occur so sporadically and at a rate so low that their detection and the determination of the rate at which the changes take place are almost, but not entirely, beyond investigation.

In *Drosophila*, for example, at a time (1925) when almost 15 million flies had been bred from known parents and fairly carefully inspected, the principal gene determining eye color had been observed to mutate 25 times. No other gene mutated so frequently. This gives a rate of 10^{-4} for the mutation frequency of a given gene per life cycle, if we allow for the fact that about one per cent of the flies are bred (Haldane, 1932). Again Haldane states that 200,000 individuals belonging to the species *Primula sinensis* have been bred at Merton and their mutations have been closely observed. One visible mutation occurs in 20,000 plants and no mutation has occurred more than once. In man, Haldane has computed that the gene for hemophilia has a mutation rate of 10^{-5} per life cycle. In *Antirrhinum* Baur (1926) reports a frequency of detectable mutations considerably higher than in other organisms examined.

It will be seen that the natural mutation frequency for any given gene is so low that the determination of the rate of change presents serious statistical difficulties. It is also clear that mutation frequency differs for the several genes in the same chromosome; it differs from chromosome to chromosome in the same species and furthermore, there are marked differences in the rate of different species. The difference in frequency between species is illustrated by the rates in *Primula, man* and *Antirrhinum* noted above; Muller and Altenburg (1919) and Muller (1923) in similar experiments in *Drosophila* obtained a ratio

TABLE 3

Natural mutations in the X-chromosome of Drosophila melanogaster which have occurred more than once, showing locus, total number of times each gene mutated (1925), and number of allelomorphs of each gene

MUTATIONS	LOCUS	TOTAL OCCURRENCES	NUMBER OF ALLELOMORPHS
Yellow	0	15	2
Scute	0	4	1
Broad	0.6	6	4
White	1.5	25	11
Notch	3.0	25	3
Ruby	7.0	6	2
Crossveinless	13.7	2	1
Cut	20.0	16	5
Singed	21.0	5	3
Tan	27.5	3	2
Lozenge	28.0	10	5
Vermilion	33.0	15	2
Miniature	36.1	7	1
Garnet	44.4	5	3
Rudimentary	54.5	15	5
Forked	56.5	12	4
Bobbed	70.0	6	1

of one mutation in 53 for the X-chromosome and one in 166 for the second chromosome. The frequency of mutations for the genes of the same chromosome are well illustrated in the following table, where the genes of the X-chromosome of *Drosophila melanogaster* are represented (Morgan, Bridges, Sturtevant, 1925). Table 3 gives what is undoubtedly the largest number of genes mutating spontaneously the greatest number of times each, in any chromosome in any species. Yet when it is considered that these numbers, even the highest (i.e., 25 for white and notch, 15 for vermilion, etc.) are based on a count of between 15 and

20 million flies, the rate is seen to be exceedingly small. And so, as Muller (1922, 1923) remarks, a mutation is a "windfall" and the expression "mutation frequency" a contradiction in terms. "A few years ago to have attempted to study the mutation rate would have been as absurd as to study the distribution of dollar bills on a sidewalk or to search for the proverbial needle in a haystack. Each mutation was a unique event."

Muller and Altenburg (1919) were at this early date studying the mutation rate in *Drosophila* and pointing out the importance of the rate of hereditary changes for an adequate understanding of evolution. They suggested that lethal mutations, which occur so much more frequently than visible changes, held the key to this problem. In these experiments the set-up was arranged in such a way that any sex-linked lethal arising in a female would kill off half of her sons, giving a 2 to 1 sex ratio. It was only necessary then to count the number of cultures having this peculiar sex-ratio to determine the number of lethal mutations. In one experiment 90 females were tested and found to be free from lethals. Breeding these for five generations, the sex-ratios of 385 families were determined and during this time 13 of the families gave the 2 to 1 sex-ratio, thus exhibiting the occurrence of a lethal gene in the X-chromosome. This work repeated on a larger scale gave 1,062 families with 20 sex-linked lethal mutations or one in 53. Accepting this as representing the normal frequency rate of mutation in the X-chromosome and assuming that mutations occur with this as an average frequency in the other chromosomes,—then since the X's form about one-fourth of the entire chromatin mass, Muller and Altenburg believe that one fly in every thirteen has a new lethal in some one of its chromosomes. It is evident, that at this rate, without natural selection to weed out the unfit the race would soon become filled with lethals. Or to put the matter differently, each X-chromosome would have one lethal after 100 generations—about four years in *Drosophila.* The importance of these figures is very great because they are the first based on accurate count of gene mutations in a chromosome, and these figures were found to be startlingly high.

This immediately indicates, say Muller and Altenburg, that *Drosophila* must have a different rate from some other species—for example, man. For if the X-chromosome of man mutated at this rate, the X-chromosomes of all the females would contain a number of lethals by the time she was ready to reproduce, and none of her sons would be viable. With a minimum number of 500 genes in the X-chromosome of

Drosophila and one mutation in this chromosome every four years, then the rate of change for each individual gene would be once in 2000 years. This is the accepted rate of change in radium, and the so-called mutable *Drosophila* is seen to have a spontaneous rate of change which is not greater.

However, there is one class of specially mutating genes which have a spontaneous rate of change so rapid that at the end of a few decades half of the genes descended from those originally present would have become changed. These are called "eversporting" genes, and the way is now open through the work of Emerson (1911), Baur (1926), Demerec (1926, 1927), and others, to arrive at a natural mutation frequency. But these are special cases. For the ordinary genes such as those of *Drosophila* listed in the above table, Muller (1922), and Patterson and Muller (1930), compute that at the normal rate of change it would take a thousand years (probably longer) before half of them mutated. This is a stability quite on a par with, if not higher than, that of radium atoms.

As a number of investigators have pointed out, it is a matter of the greatest importance to be able to determine the rate of mutation, both under natural and under artificial conditions, and also to be able to make an exact comparison between the two rates (Patterson, 1929). Morgan (1932) says that "If it can be shown on a large scale that the same ratio for known mutations holds for x-ray and for spontaneous mutations, we may have found an opening for the further study of the causes of certain types of mutation."

Since by means of x-rays and radium it is possible to increase the rate of mutation for certain genes up to 15,000 per cent above the normal (Muller 1928, 1929), it is now possible for the first time to ascertain with something approximating accuracy the mutation frequency for any given gene and to make a comparison of this rate with that of other genes similarly treated and with that of untreated genes. A start already has been made and Hanson and Miller (unpublished) are engaged in an extensive experiment for the purpose of comparing the rate of change of certain sex-linked genes in normal and x-rayed flies.

Patterson and Muller (1930) have combined results of experiments done in their laboratory and have shown that following heavy x-irradiation the locus for white-eye in *Drosophila* has exhibited the highest rate of mutation among the several genes. The combined results indicate that the change from red to white arises in something like one in 1000 X-chromosomes. This is also the locus in *Drosophila* at which

the greatest total number of "spontaneous" mutations have been detected. There are also a dozen or more different mutant allelomorphs of spontaneous origin known at this same locus, and these appear after raying also. The actual mutation frequency figures for this locus as computed by Patterson and Muller (1930) are, for the spontaneous rate: in the case of white: almost one in 400,000 times, and, in the case of the other allelomorphs: about one in 600,000 times. The induced mutation frequency in round numbers was: about one in 1800 sperm cells for white, and about one in 23,000 sperm cells for the allelomorphs of white. The frequency of appearance of mutations at the white locus in the irradiated sperm was of the order of magnitude of 200 times the corresponding frequency in non-radiated material. If, however, the flies in all the experiments are weighted according to the dosage of radiation used—which is legitimate, in view of the direct proportionality between dosage and the mutation rate (Hanson and Heys, 1929),—there would have been one detectable mutation of the normal allelomorph of white to one of the mutant allelomorphs of white among about 1,000 sperm cells treated with the heaviest of the dosages used. This result is to be compared with a frequency of one in about 350,000 in the non-radiated material, an increase in the frequency of treated sperm cells of about 35,000 per cent.

In the case of the locus for "scute"—the radiation frequency of mutation is about 250 times that of the normal; the mutation frequency of one of the allelomorphs of "forked" found in the treated is over 300 times that found in the untreated lines. Such enormous differences between the natural and induced rate of mutation frequency renders most highly probable the view that in these cases radiation was the causative agent of the mutation increase.

Hanson and Heys (1928) and Oliver (1930) have shown for radium and x-rays, respectively, that the rate of mutation in *Drosophila* is determined by the strength of the dosage applied. The rate seems to be directly proportional to the dosage. Muller has named this the "proportionality rule." For example when all other factors are kept constant, doubling the time of exposure also doubles the number of lethal mutations.

In view of these results Patterson (1931) raised the question as to what would happen if the dosage were given in fractions at regularly spaced intervals, instead of continuously as a single dose. That is, would the summation of several spaced treatments give a cumulative effect on the rate of mutation, or would the rate be the same as for a

single treatment in which the total dosage was used? Patterson used both x-rays and radium and found that the rate of mutation, as measured by the number of sex-linked lethals produced, was the same whether the treatment was given in one dose or in several fractional doses, provided the total amount of radiation under each of the two conditions was equal. As illustrative of Patterson's results in the experiment with radium one group of flies was exposed continuously for twelve hours, while the other group was treated with twelve exposures of exactly one hour at regular intervals of twelve hours. The F_2 cultures gave for continuous and spaced treatment 10.44 per cent and 10.40 per cent of lethals, respectively. The difference is obviously not significant. Serebrovsky and Dubinin (1930) report that, "It is clear that lengthening the time of exposure with interruption or without raises the per cent of mutation." They do not state whether the percentages were the same or different if the two methods under which the dose was given, were compared.

Harris (1929) has compared the mutation frequencies when sperm of different ages were irradiated. He finds the percentages of mutations almost identical whether the treated mature spermatozoa were of the age zero to four days, four to eight days, or sixteen to twenty days,—after raying—and concludes that "the mutational effect of x-rays upon the mature sperm probably is permanent during the life of the sperm."

However, Harris and also Hanson and Heys (1929) independently found that in the case of males which are continuously mated the frequency of mutation is five to ten times greater in offspring produced during the first twelve or fourteen days after raying, than during a second period of like duration. This indicates a difference in rate of mutation as between mature spermatozoa and germ cells in the early stages of spermatogenesis.

Patterson (1929) carried out extensive experiments on induced mutations in somatic cells in fruit flies and has computed a gene frequency of mutation of one in about every 10,000 genes. Chromosome breakages also take place in somatic cells following irradiation and the rate of breakage is one in about every 713 X-chromosomes.

Breakages in chromosomes are often followed by the attachment of a broken particle of a chromosome to one of the other chromosomes. This is called a translocation, and important problems are in process of being solved through studies, both genetic and cytologic, of these translocated pieces of chromosomes. Muller and Altenburg (1930)

have especially interested themselves in the frequency of translocations following x-radiation, and have found that the rate of occurrence of translocations may be just as high, or higher than that of gene mutations. This is a surprising result in view of the fact that in all previous work with *Drosophila* only two cases of natural translocations had been found (Bridges, 1923; Stern, 1926), while some 400 natural gene mutations had accumulated. Since translocations are usually accompanied by lethal or other detectable phenotypic effects, a large number of them should have been found if natural translocations were actually as numerous as natural gene mutations. We must conclude that this is not the case. How then shall we reconcile these two conflicting series of findings? Muller and Altenburg first point out that the natural cases dealt with represent the sum total of all mutations that have occurred in both sexes and at all different stages in the life cycle, while practically all induced gene mutations and translocations have occurred at one stage in one sex—the mature spermatozoa. It has already been pointed out that this is a particularly sensitive stage of the chromatin. But more important is their conclusion that there is a real difference between x-rays and the "natural" cause or causes of mutations, in regard to their relative effectiveness in altering the genes or causing breakages and reattachments of parts of chromosomes. This latter conclusion is strengthened by the results of Muller and Mott-Smith (1930), indicating that natural radioactivity is not the cause of most mutations in nature.

Patterson (1932) obtained 230 cases of mutations in the X-chromosome of *Drosophila* following treatment with x-rays. These were distributed among 15 genes in that chromosome, and included point mutations, both visible and lethals. The lethals were mostly due to slight deficiencies, varying in extent from cases involving a single locus to cases in which several adjacent loci were affected. The number of mutations occurring at the different loci varies greatly from single cases up to 53 at the locus miniature. However, if the cases at "white" and "white-notch" are added together, the total is 65 for this locus. It may be recalled, in this connection, that the locus for "white" also has given the largest numbers of natural mutations. The ratio of lethal to non-lethal mutations was approximately seven to one, again paralleling natural mutations. The actual rate of mutation frequency was not determined as this would have involved counting all the thousands of unaffected flies.

Hanson and Heys (unpublished) treated the eggs of *Drosophila* with

polonium rays. These eggs were treated two and one-half hours after they were laid. They were, at this age, in the blastula stage. Six hundred and seventy-five such eggs were exposed to the same dosage of polonium and from these 203 flies hatched. All were abnormal in one or several of their external characters. Breeding tests gave all perfectly normal offspring. This constitutes 100 per cent of mutations in somatic cells. Somatic mutations have been described above on the basis of the work of Patterson who used x-rays for treatment of eggs and larvae of different ages.

Stubbe (1932) compared the normal gene mutation rate in *Antirrhinum* with the rate after exposure to Grenz-rays and x-rays. The average of many doses of Grenz-rays gave an average increase in the mutation rate of 300 per cent; x-rays increased the rate about 400 per cent within the voltage range from 20–70 K.V.P. A relation between the quality of the rays and the mutation rate occurred only in the range from 30–50 K.V.P. Stubbe thinks that within this range conditions of absorption are more favorable and hence more mutations are produced than by harder or softer rays. Stadler (1928, 1930, 1931), Zeleny (1921), and Muller (1928), have studied the effect of temperature upon the mutation rate, as well as Goldschmidt (1929), who induced mutations in *Drosophila* by heating the eggs to such a degree as to kill most of them. The mutations obtained among the surviving eggs were, unlike those obtained by raying, not occurring at random, but composed largely of two types, characterized by a dark body and an abnormally veined wing. Jollos (1930) confirmed and extended the results of Goldschmidt, but Rokizky (1930) was able to confirm Goldschmidt and Jollos only in part, while Szidorov, Ferri and Shapiro (1929) repeated the experiments with negative results.

Zeleny (1921) found no difference in the mutation rate of bar-eye between stocks kept at 20°C. and those kept at 27°C. Stadler did not obtain significant differences in the rate of mutation in either dormant or germinating barley seed when exposed to temperatures ranging from 10°C. to 50°C. Stadler also combined variations in temperature with radiation. He found that seeds imbedded in solid carbon dioxide (temperature of sublimation:— 80°C.) would tolerate radiation intensities about two-thirds higher than those tolerated by dormant seeds at ordinary temperatures. But the difference in mutation frequency between the two temperatures was not a significant one. Again germinating seeds were irradiated in intermittent exposures at 30-minute intervals through a period of seven and one-half hours. The variation

in temperature range from 7°C. to 37°C. was without an appreciable effect on the mutation rate; neither was the mutation rate affected when temperatures varying from −80°C. to 38°C. were applied after irradiation instead of during it.

On the other hand, Muller (1928) has been successful in inducing mutations by heat in flies. His paper published in 1928 reviews work extending over a period of ten years along this line, with many near successes and many struggles to build up special stocks for attacking the problem as to the mutation frequency induced by means of temperature. At last, with improved breeding methods and better physical equipment Muller obtained positive results. In his "cold series" kept at 19°C., 381 lines survived to be tested. These represented 6,286 "chromosome generations," or 4,191 "chromosome-months." Among these, 12 lines were found to contain a lethal mutation. This is a rate of one lethal in 524 chromosome-generations, or 0.19 per cent of mutation per generation for the X-chromosome. The time rate was 1 in 349 chromosome-months, or 0.29 per cent.

In the warm series kept at 27°C. there were 359 surviving lines which represented 6,462 "chromosome-generations," or 4,308 chromosome months. The number of lethals was 31, with a mutation rate, per chromosome generation, of one in 208, or 0.48 per cent. The time was one in 139 chromosome-months, or 0.72 per cent. The difference between the mutation rates, per chromosome-generation, of the cold and warm series, was 0.29 per cent, which is 4.1 times its probable error. Such a difference would have only one chance of occurrence in 195 experiments, or one in 390 if direction of changes to a specific mutation is considered. The effect may therefore be regarded as proved, in the sense of having that high degree of probability which passes as proof in much scientific work.

These results constitute the first demonstration of the effectiveness for any specific agent whatever in influencing the mutations of numerous genes and as such set up a landmark in genetic progress. As Muller points out we do not know how direct the effect of temperature is in producing mutations but we do know that in the case of ordinary chemical reactions the direct effect of a rise in temperature is in the direction of an increased speed of the reactions, the magnitude of the effect being about 100 to 200 per cent for each 10 degrees Centigrade within the range of ordinary temperatures. The mutation rate, following treatment with heat, is in the same direction and the magnitude of effect is of the same order. These facts suggested that mutation is primarily of the nature of a chemical change.

It was the very next year (1927) that Muller first announced success in speeding up the mutation rate about 15,000 per cent by the use of x-rays. This figure is to be compared with the somewhat less than 100 per cent increase with rising temperature. Hanson (1928) followed with similar results from radium rays, Weinstein (1928) and others obtained confirmatory results with the x-ray. Thus two external agents, heat and radiation, have been established at the present time as affecting the spontaneous mutation rate. There are undoubtedly other such agents. For as Muller and Mott-Smith (1930) have shown, natural radiation is insufficient to account for natural mutations. We must conclude that there are probably a number of "causes" of mutations in nature of which only temperature and radiation are definitely known. No one cause then can be said to be specific. There is a trigger that needs to be touched off. What we now need most to know is, what are the "natural triggers"—i.e., what are the causes of hereditary changes in organisms in nature. With the exception of our knowledge as to the feebly acting temperature and the strongly acting radiation, we have not much advanced from the days of Darwin who said of the causes of variability "The subject is an obscure one; but it may be useful to probe our ignorance."

Tice (1914) discovered the mutation from full-eye to bar-eye. It has been reported only once. However, May (1917) demonstrated that the change from full to bar is reversible and obtained eleven such reversions, a mutation rate of about once in 1,600 times. (See section on "progressive mutations" for a discussion of the bar-eye work by Zeleny (1921) and Hanson (1928).)

Stadler (1929) has made important contributions to our knowledge of mutation rates in barley and oats. In barley, after irradiation of the dormant seeds, mutations have occurred at rates exceeding 5 per cent, and the rate of mutation was exactly proportional to radiation intensity.

The mutation rate varies from species to species and from gene to gene in the same chromosome. Why two genes lying next to each other on the chromosome should exhibit widely varying mutation frequencies is one of the important unsolved problems of radiation-genetics.

However the problem as to the cause of the variation in mutation rates between different species seems to have been solved for certain species by Stadler. He found that the same dose of radiation that gave as many as 5 per cent mutations in barley induced few or no mutations in oats. Why this difference between barley and oats?

The species of common barley used by Stadler has seven chromosomes. The two common species of oats (*Avena byzantia* and *A. sativa*) have 21 pairs of chromosomes and are considered to be polyploid species. That is, it may be assumed that the triple complement of 21 pairs of chromosomes represents a combination of three groups of seven pairs each, and that these three groups are identical in at least some of their genes. In *Triticum* there are species with 7, 14 and 21 pairs of chromosomes. In polyploid species with duplicate genes if a mutation is induced in one of the two or three duplicate genes present no visible effect could result because the unmutated genes would produce the normal character. This would not hold for dominant mutations but they are relatively infrequent in occurrence.

In Stadler's experiment with *Avena,* he rayed four species (two having 7 chromosomes each and two 21 each) giving them a dose that in barley would have yielded 70 mutations. The two species of *Avena* with 7 chromosomes yielded 14 mutations, the two with 21 chromosomes, none. In the experiments with *Triticum,* four species were treated, one with 7 chromosomes, two with 14 chromosomes and one with 21 chromosomes. The rate in the 7-chromosome species was not significantly different from the high rate obtained in barley; that in the two 14-chromosome species was markedly lower and the 21-chromosome species yielded no mutations.

The results as a whole support the view that the frequency of induced mutation observed in polyploid species is low or entirely absent because of gene reduplication. While other factors may also affect the rate, it is clear that in the species of both *Avena* and *Triticum* the mutation rate decreases sharply with increasing chromosome number.

In maize, Stadler (1930) counted a million and a half seeds. Seven out of eight of the normal genes studied mutated at least once, and one gene had a mutation rate of about 4×10^{-4} per generation. Stadler (1928) was not successful in getting significant increases or decreases in the cross-over frequency of the genes in maize by means of x-rays. There was, however, a pronounced increase in the percentage of mosaic endosperms following x-ray treatment. There is also a distinct difference between dormant and germinating seeds. The latter have a mutation frequency of about eight times the former for the same dosage. On the other hand dormant seeds tolerate a dose 15 to 20 times as great as that tolerated by germinating seeds.

Stadler (1928, 1930, 1931) treated pollen of maize and observed chromosome irregularities. The frequency of the deficiencies induced

by irradiation of the pollen was directly proportional to dosage for the five chromosomes investigated. For the individual genes the frequencies of "loss" per 1000 r varied from 2 per cent to 6 per cent.

About 1600 seedling mutations in barley have been recorded by Stadler. Dominant mutations are extremely rare and it is possible that no dominants have occurred; however there were four possibilities and these have not been genetically tested. This extreme rarity (or possibly even complete absence) of induced dominant mutations leads Stadler to speculate that possibly induced mutations are a special class of mutation, i.e., while a 100-fold increase in the total rate may mean that mutations in general are increased 100-fold, it may also mean that mutations of a type comprising, say, 10 per cent of the total are increased 1000-fold. On the other hand, Stadler's visible mutations induced by irradiation are identical with typical mutations of spontaneous origin. This is also true even to a greater extent in *Drosophila*, with the added fact that dominant mutations in the fly are not of rare occurrence (Muller, 1930; Van Atta, 1932, 1932a).

Karper (1932) describes a dominant gene mutation in sorghum. This gene change adds about 40 per cent to the height of the plants, but does not increase the number of internodes. Eight inbred lines produced a total of 14,520 plants in the period 1927–31, and among them appeared 24 separate mutations for "tall." The mutation ratio, considered on the basis of zygotes, is 1 to 604. Presuming that the mutation of this gene may occur as readily among the male gametes as among the female, the mutation ratio in the gametes is 1 to 1,209, or 827 per million. This is about the highest rate of spontaneous mutation known in plants. As the dominant mutants lead to an increased productivity and are superior from the standpoint of forage and silage, they can be, according to Karper, definitely classified as favorable mutations in the biological sense. (See also section on "progressive mutations" in this paper for other cases of progressive changes.)

CONCLUSION

Muller's discovery of the effectiveness of radiation in producing mutations is a good example of the fact that the discovery of a new tool or a new method in science is often of greater importance than any one scientific observation or experimental result. For Muller has put in the hands of geneticists and other biologists a method for attacking many problems, both old and new. Already important experimental results have been obtained and the possibilities are as yet but scarcely touched.

BIBLIOGRAPHY

BABOCK, E. B. AND J. L. COLLINS. 1929. Proc. Nat. Acad. Sci., xv, 625.
BAGG, H. J. AND C. C. LITTLE. 1924. Amer. Journ. Anat., xxxiii, 119.
BAUR, E. 1926. Zeitschr. indukt. Abstamm., xli, 47, 251.
BERGONIÉ, J. AND L. TRIBONDEAU. 1906. Compt. Rend. Acad. Sci., cxliii, 983
BRIDGES, C. B. 1923. Anat. Rec., xxiv, 426.
BRINK, R. A. AND P. H. SENN. 1931. Journ. Hered., xxii, 155.
BROADFOOT, W. C. 1932. Science, lxxv, 334.
CONTAMIN, M. A. 1909. Compt. Rend. Acad. Sci., cxlix, 1397.
1910. Compt. Rend. Acad. Sci., cl, 1537.
CANTI, R. G. AND M. DONALDSON. 1926. Proc. Roy. Soc. B., c, 413.
CURTIS, W. C. 1928. Science, lxvii, 141.
DEMEREC, M. 1926. Proc. Nat. Acad. Sci., xii, 11, 687.
1927. Proc. Nat. Acad. Sci., xiii, 249.
DEMEREC, M. AND J. G. FARROW. 1930. Proc. Nat. Acad. Sci., xvi, 711.
DUNCAN, F. N. 1915. Amer. Nat., xlix, 575.
EMERSON, R. A. 1911. Amer. Nat., xlviii, 87.
GOLDSCHMIDT, R. 1929. Biol. Zentralbl., xlix, 473.
GOODSPEED, T. H. 1932. Svensk Botanisk Tidskrift, xxvi, 147.
GUYÉNOT, E. 1914. Bull. Sci. France Belg., xlviii, 160.
HAENDLY, P. 1918. Arch. f. Gynaek., cix, 409.
HALDANE, J. B. S. 1929. Nature, cxxiv, 514.
1932. Harper Bros., New York.
HANCE, R. T. 1928. Journ. Hered., xviii, 377.
HANSON, F. B. 1928. Amer. Nat., lxii, 352.
1928. Science, lxvii, 562.
HANSON, F. B. AND F. HEYS. 1928. Science, lxviii, 115.
1929. Amer. Nat. lxiii, 511, 201.
1930. Science, lxxi, 43.
1932. Amer. Nat., lxvi, 335.
1933. Amer. Nat., in press.
HANSON, F. B. AND F. R. FERRIS. 1929. Journ. Exper. Zool., liv, 485.
HANSON, F. B. AND E. WINKLEMAN. 1929. Journ. Hered., xx, 277.
HARRIS, B. B. 1929. Journ. Hered., xx, 299.
HARRISON, J. W. H. 1928. Proc. Roy. Soc., B, cii, 347.
HAYES, H. K. AND H. E. BREWBAKER. 1924. Journ. Hered., xv, 497.
HERTWIG, P. 1911. Arch. f. Mikr. Anat., lxxvii, 301.
HORLACHER, W. R. AND D. T. KILLOUGH. 1933. Amer. Nat., in press.
HUGHES, A. W. K. 1931. Nature, cxxviii, 496.
JANNSON, G. 1927. Acta Radiologica, viii, 427.
JOLLOS, V. 1930. Biol. Zentralbl., l, 541.
JOLY, J. 1929. Nature, cxxiv, 579.
JOLY, J. AND H. H. DIXON. 1929. Nature, cxxiii, 981.
KARPER, R. E. 1932. Amer. Nat., lxvi, 511.
KRAFKA, J., JR. 1920. Journ. Gen. Physiol., ii, 409.
LAZARUS-BARLOW, W. S. AND H. BECKTON. 1913. Arch. Middlesex Hosp., 12th Cancer Report, 47.
LINDSTROM, E. W. 1925. Genetics, x, 442.

LOEB, J. AND F. W. BANCROFT. 1911. Science, n.s., xxxiii, 781.
MANN, M. C. 1923. Journ. Exper. Zool., xxxviii, 213.
MAY, H. G. 1917. Proc. Nat. Acad. Sci., iii, 544.
MORGAN, T. H. 1911. Science, n.s., xxxiii, 496.
1914. Amer. Nat., xlviii, 705.
1914. Pop. Sci. Monthly, xiv, 5.
1932. Science, lxxvi, 261, 285.
MORGAN, T. H., C. B. BRIDGES AND A. H. STURTEVANT. 1925. Biblio. Genetica, ii, 1.
MOTTRAM, J. C. 1913. Arch. Middlesex Hosp., 12th Cancer Report, 98.
MULLER, H. J. 1922. Amer. Nat., lvi, 32.
1923. Anat. Rec., xxvi, 397.
1923. 2nd. Internat. Congress Eugenics, Vol. i.
1923. Anat. Rec., xxiv, 419.
1926. Proc. Internat. Congress of Plant Sciences, i, 897.
1927. Science, lxvi, 84.
1928. In. Verhandlungen des V. Internationalen Kongresses für Vererbungswissenshaft, Berlin, 1928, 234.
1928. Genetics, xiii, 279.
1928. Proc. Nat. Acad. Sci., xiv, 714.
1928. Science, lxvii, 82.
1929. Smithsonian Report, 345.
1929. Sci. Monthly, xxix, 481.
1930. Journ. Genetics, xxii, 299.
1930. Amer. Nat., lxiv, 220.
MULLER, H. J. AND E. ALTENBURG. 1919. Proc. Soc. Exp. Biol. Med., xvii, 10.
1928. Anat. Rec., xli, 100.
1930. Genetics, xv, 283.
MULLER, H. J. AND L. M. MOTT-SMITH. 1930. Proc. Nat. Acad. Sci., xvi, 277.
MULLER, H. J. AND T. S. PAINTER. 1929. Amer. Nat., lxiii, 193.
NORTHROP, J. H. 1920. Journ. Gen. Physiol., ii, 313.
OLIVER, C. P. 1930. Science, lxxi, 44.
OLSON, A. R. AND G. N. LEWIS. 1928. Nature, cxxi, 673.
ORTON AND BURFIELD. 1931. Nature, cxxviii, 376.
PAINTER, T. S. AND H. J. MULLER. 1929. Journ. Hered., xx, 287.
PATTERSON, J. T. 1928. Science, lxviii, 41.
1929. Journ. Hered., xx, 260.
1929. Journ. Exper. Zool., liii, 327.
1930. Anat. Rec., xlvii, 394.
1930. Proc. Nat. Acad. Sci., xvi, 109.
1931. Biol. Bull., lxi, 133.
1932. Amer. Nat., lxvi, 193.
PATTERSON, J. T. AND H. J. MULLER. 1930. Genetics, xv, 495.
PAYNE, F. 1911. Biol. Bull., xxi, 297.
PLOUGH, H. H. 1917. Journ. Exper. Biol., xxiv, 147.
1932. Amer. Nat., lxvii, 83.
PLOUGH, H. H. AND M. B. STRAUSS. 1923. Journ. Gen. Physiol., vi, 167.

REGAUD, C. 1922. Compt. Rend. Soc. Biol., lxxxvi, 787.
1923. Bull. de l'Assoc. francaise pour l'Etude du Cancer, xii, 482.
REGAUD, C., A. LACASSAGNE ET J. JOVIN. 1925. Compt. Rend. Soc. Biol., xciii, 1587.
ROKIZKY, P. T. 1930. Biol. Zentralbl., l, 554.
SEREBROVKSY, A. S. AND N. P. DUBININ. 1930. Journ. Hered., xxi, 259.
SSIDOROV, B., L. FERRI AND N. SHAPIRO. 1929. Science and Marxism. 4. (quoted by ROKIZKY.)
STADLER, L. J. 1928. Science, lxviii, 186.
1928. Proc. Nat., Acad. Sci., xiv, 69.
1928. Anat. Rec., xli, 97.
1929. Proc. Nat. Acad. Sci., xv, 876.
1930. Anat. Rec., xlvii, 381.
1930. Journ. Hered., xxi, 1.
1931. Sci. Agric., xi, 645.
1931. Sci. Agric., xi, 557.
STERN, C. 1926. Biol. Zentralbl., xlvi, 505.
STRANGEWAYS, T. S. P. AND F. L. HOPWOOD. 1926. Proc. Roy. Soc., B., c, 283.
STUBBE, H. 1932. Zeitschr. f. Ind. Abstammungs. u. Vererb., lx, 474.
STURTEVANT, A. H. 1925. Genetics, x, 117.
TICE, S. A. 1914. Biol. Bull., xxvi, 221.
TIMOFEEFF-RESSOVSKY, N. W. 1929. Amer. Nat., lxiii, 118.
1931. Ergebn. d. Med. Strahlenforschung, v, 131.
VAN ATTA, E. W. 1932. Amer. Nat., lxvi, 93.
VAN ATTA, E. A. 1932. Genetics, xvii, 637.
VINTEMBERGER, P. 1928. Compt. Rend. Soc. Biol., xcviii, 532.
WEINSTEN, A. 1928. Science, lxvii, 376.
WHITING, P. W. 1929. Journ. Hered., xx, 269.
1932. Genetics, xvii, 1.
ZELENY, C. 1921. Journ. Exper. Zool., xxxiv, 203.

THE AUTONOMIC INNERVATION OF THE GENITO-URINARY SYSTEM

CHARLES M. GRUBER

Department of Pharmacology, Jefferson Medical College, Philadelphia, Pa.

In this review of the autonomic innervation of the genito-urinary system I shall adhere as closely as possible to the nomenclature used by Langley (17) in his excellent book.

Although the vagus sends a few fibres to the kidneys, in the main the urogenital system is supplied by the thoracico-lumbar autonomic or sympathetic and the sacral autonomic a branch of the parasympathetic systems. The sympathetic nerve supply of the body arises from the spinal cord from the origin of the first thoracic and the 2nd, 3rd or 4th lumbar nerve roots—the lowest nerve varying slightly in different animals and more widely in different species. The genito-urinary system, however, receives fibres only from the 4th thoracic to the 6th lumbar segments. These nerves possess a white ramus communicans containing pre-ganglionic fibres, which are efferent in function and before reaching their effector organ meet a cell-relay and form a synaptic junction with cells contained either in one or more of the lateral chains of sympathetic ganglia, or in some part or parts of the collateral pre-vertebral chain. In general each pre-ganglionic fibre is connected by means of collateral branches with several ganglionic cells in the sympathetic chain, but that between any connector cell in the central nervous system and the effector organ in the periphery one and only one cell station is intercalated, i.e., broadly speaking, each sympathetic ganglion is a cell station for all classes of visceral fibres which supply a definite area. In all cases every spinal nerve after leaving the spinal cord receives an accretion of fibres from the lateral sympathetic chain of ganglia as a grey ramus containing post-ganglionic effector fibres and to this system belong the motor nerves for the muscles of the blood vessels of the whole body; in some instances inhibitory, in others motor nerve fibres to the entire genito-urinary system.

Concerning the general problems of the sacral autonomic nervous supply investigations have shown that, physiologically speaking, the out

flow of efferent visceral fibres is the same as that of the bulbar system. It supplies, as a rule, motor control to a single system which may be spoken of as endodermal (2). The connector cells of this system whose axons form long connector fibres to the peripherally placed ganglion cells all lie inside the central nervous system, while the effector post-ganglionic fibres are extremely short, the ganglionic cell itself lying close against the musculature which it supplies. The sacral or pelvic nerves do not pass into well defined ganglia, but pass instead, as a rule, into a nerve-plexus which may extend over the surface of the organ, e.g., the bladder and rectum. In this way it may be divided roughly into vesical and rectal portions. From these plexuses post-ganglionic effector fibres pass to supply the genito-urinary system and other organs.

In spite of the large amount of experimental work which has been done on the investigation of the autonomic innervation of the genito-urinary system much yet remains to be learned. This may be attributed to two main factors. The first of these is the difficulty in getting clear cut results, independent of outside influences, and the second the fact that these influences make the experimental data thus obtained subject to many interpretations according to the fancies of the investigator.

Langley (3) claims that the documents written on physiology before 1860 are little more than formulae which had been handed down from generation to generation. This is true to a certain degree of the work on the genito-urinary system. The early writers followed very distinct channels, yet even some of the modern writers advance theories as being new and original which are really old ones couched in different terms.[1] In spite of the lack of experimental research, as early as 1559 Vesalius (4) described the autonomic innervation of the uterus in a quite modern manner. The fundus uteri, he wrote, is innervated by the sympathetic and the cervix uteri and lower uterine segment are innervated by the sacral nerves. Valentin (5) in 1839 described contraction of the urinary bladder and uterus upon stimulation of the upper sacral and lower lumbar nerve roots.

KIDNEY. The kidney is supplied with nerves by way of the renal plexus, which arise from the semilunar ganglion through which it communicates with the greater and lesser splanchnic and the vagus nerves

[1] Upon reading the literature some differences in opinions will be noted. Gaskell recognizes but five lumbar vertebrae whereas Bradford believes there are seven. In both cases the 21st spinal nerve is the 13th dorsal, but Gaskell calls the 25th spinal nerve the 2nd lumbar whereas Bradford considers the 23rd as the 2nd lumbar and the 25th as the 4th lumbar.

(6). It also usually receives a branch direct from the lesser splanchnic and the first lumbar ganglion (7), and according to Pawlinko (8), the splanchnic minimums, a small branch which is seldom found, runs from the last sympathetic ganglion to the renal plexus. The splanchnic major in turn is formed mainly by fibres from the 6th to the 12th dorsal nerves, although some fibres are found as high as the 4th dorsal and some as low as the 3rd and 4th lumbar nerves (9).

That the splanchnic major supplies only a fraction of the sympathetic nerve supply to the kidney has been demonstrated clearly by Hirt (10). He believes, anatomically the nerves of the kidney can be collected into four groups, splanchnic major, splanchnic minor, lower abdominal sympathetic and the vagus. If the vagus is omitted, physiologically they can be collected into two groups, the splanchnic major and lower abdominal sympathetics, which are antagonists. According to Hirt, the splanchnic major originates from eleven thoracic ganglia and goes to the splanchnic ganglion where it gives off a branch to the coeliac ganglion and one to the kidney. The splanchnic minor, originating from the 13th thoracic to the 2nd lumbar segment he divided into four separate nerve trunks of which the upper branch is intimately bound up with the splanchnic major trunk and the lower with the lower abdominal sympathetic. The middle two branches of this nerve pass directly to the kidney without terminating in ganglia. These he believes are direct spinal fibres to the kidney and are probably sensory in type. The abdominal sympathetic he states originates from the 2nd and 3rd lumbar segments. These leave the spinal cord in the anterior roots and are interrupted for the first time in the formation of a plexus. They then combine with the aortic plexus and by further anastomosis part of the fibers pass to the renal ganglion and from here post-ganglionic fibres pass to the kidney.

Hirt (64) believes the difference in opinions on the vagal innervation of the kidney can be explained upon anatomical grounds. In the cat he found that only the posterior right vagus passes to the coeliac plexus whereas the left innervates the stomach. The same is true in the rabbit. In the dog there are multiple anastomoses of both vagi even though only the posterior right branch passes to the coeliac ganglion. In man he finds the anastomosis of the two vagi too rich to speak of them as separate nerves, although again the posterior branch of the right vagus appears always to pass to the coeliac ganglion. The assumption that the two vagus nerves innervate the kidneys homolaterally is apparently incorrect.

A combination of fibres from these main nerves makes up the renal nerve trunk. Many of these nerve fibres anastomose and form what is called the renal plexus with its ganglia. That there is still much disagreement between investigators on the anatomical findings and the question of presence of ganglia can be seen from the fact that according to Pappenheim (11), Hoebler (12), Hrynstschak (13), and Schilf (14), no ganglia exist in either the kidney pelvis or in the kidney parenchyma. While, on the other hand, with equally good technique, ganglia have been described in the kidney pelvis by Maier (15), Stöhr (16), and Hirt (10). Hoebler (12) agrees that ganglia may be found in the kidney pelvis in young individuals but he has been unable to show them in the adult human kidney. The fibres of the renal plexus are arranged in a network about the vessels of the renal pedicle. These fibres enter the kidney with the renal vessels and follow them to their finest ramifications, small fibrils being found in the smallest afferent and efferent vessels even on the capillaries. Nerve fibrils have been described on and between the cells of the tubules and the capsule. The nerve fibres of the parenchyma of the kidney are usually non-medullated while in the pelvis and calyxes they are frequently medullated.

Pappenheim (11) was the first to describe the presence of nerve fibres in the parenchyma of the kidney and he was able to trace them as far as to the arteries of a caliber of 0.03 mm. diameter. He was unable to demonstrate ganglia in this area. Kölliker (17) could follow similar nerve fibres to the finest capillaries and he found nerve fibres forming plexuses innervating the vascular musculature and terminating on the glomerulus. His findings were confirmed by Retzius (18). This work was repeated and extended by Smirnow (19) who traced nerve fibres into the smallest capillaries, the vasa afferentia and vasa efferentia. In all of these he believed the smooth muscles were supplied with motor nerves. Some branches of these nerve fibres reached the tubules forming plexuses around them. The fibres from these plexuses ended partially on the membrana propria and partially entering the epithelium and ending between the epithelial cells. He not only noted many fine nerve fibrils in the glomerular vessels, but also in the glomerular membrane. Sensory end organs were observed in the kidney hilus and capsule and he concluded that the kidney parenchyma is innervated by secretory as well as sensory nerve fibres. His work was repeated by Disse (20) working on the kidneys of mice. He demonstrated the presence of these plexuses in the kidney but he was unable to decide whether the nerve fibre ended in the epithelium of the tubules or on the membrana propria. Similar

findings were recorded by Hirt (10) and by Kaufman and Gottlieb (21) who saw an extensive non-medullated innervation of the parenchyma of the kidney in addition to the purely vascular nerve supply. In all instances the nerve fibres apparently end as bare nerve endings since no one has described special end organs for them.

Vagus nerve. From the large amounts of accumulated evidence there can be little doubt that the vagus innervates the kidney (22, 23, 10). The function of the vagus nerve, however, still remains a mystery. The published results are extremely conflicting. Some workers believe they have been able to demonstrate vasomotor fibres in these nerve trunks (24, 25, 26, 27, 28, 29), while others, equally competent investigators, have been unable to demonstrate this function in the nerve (9, 30, 31, 32, 33, 34). In addition to this vasomotor phenomenon some investigators have attributed to the vagus secretory nerve fibres similar to those of the chorda tympani for salivary secretion, while still others attribute to it an inhibitory secretory factor.

According to Thompson (35), Walti (36) Lazzaro and Pitini (37), and others, atropine inhibits the secretion of urine and acts directly upon the secretory nerve fibres contained in the vagus. This view was immediately opposed by Eichelberg (38), Loewi (39), and Cow (40) who pointed out that the action of this alkaloid on the kidney is not so characteristic as on other true glands.

Splanchnic nerve (sympathetic). Bernard (41) observed a diuresis in a kidney of which the greater splanchnic nerve had been severed. His findings have been confirmed by Eckhardt (31), Knoll (42), Klecki (43), Vogt (44), Grek (45), Rhode and Ellinger (46), Jungmann and Meyer (47), Gubergritz (48), and others. Cyon and Benzol and Benson demonstrated an accompanying increased blood flow through the kidney following severing of the splanchnic nerve and it is now a well known fact that if the peripheral end of the cut splanchnic nerve is stimulated the volume of the kidney quickly diminishes. This was first established by Cohnheim and Roy (49) and later confirmed by Burton-Opitz (34), Bayliss and Fee (50), Renner (51), Schilf (14), Stöhr (16), Langley and Dickinson (512), and Milliken and Karr (52). Burton-Opitz (34) also noted, following such excitations, vaso-constriction of the opposite kidney. This phenomenon always occurred after a long latent period which the author later proved to be due to the liberation of epinephrine from the suprarenal gland and not to a crossed innervation of the kidney by the splanchnic nerve as other investigators were led to believe. In this manner he was able to confirm and establish more firmly his view of

homolateral innervation of the kidney by the splanchnic nerves. His observations have been confirmed by Tournade and Hermann (53). Langley and Dickinson noted few if any splanchnic vaso-constriction fibres ending in the solar plexus. They obtained evidence that these fibres for the kidney end in the renal plexus.

A difference in response of the kidney upon varying the rate of stimulation of the splanchnic nerve was observed by Bradford (9). When the rate of interruption was 50 times per second, and certainly not less than 5 times per second, the vaso-constrictor fibres were excited since such excitation was always followed by a decrease in the volume of the kidney and a simultaneous decrease in the secretion of urine. If, however, the rate of stimulation was 1 per second or 1 per 2 seconds, then the volume of the kidney increased and an increase in the secretion of urine was recorded. These observations have been confirmed by Biedl (54), Block (55), and Milliken and Karr (52). It appears therefore that the sympathetic carries not only vaso-constrictor fibres to the kidney but also vaso-dilator fibres as well, and the sympathetic system may be considered as being a mixture of nerves (510) containing both motor and inhibitory fibres running in the same nerve trunk. In the light of recent findings this is not unusual and many authorities believe that the tonus of the vessels in the organ at the time of stimulation determines the response which will be obtained.

Bunch (56) describes two effects upon the intestine on splanchnic stimulation. If we assume that epinephrine produces the same changes as sympathetic stimulation (57) then the work of Cannon and Lyman (58) and others is of significance here. A fall or a rise in the blood pressure following the injection of epinephrine was obtained by them. This was dependent upon the amount of epinephrine injected and the original control height of the blood pressure. When the blood pressure was low even a small dose of epinephrine would cause a rise whereas if the blood pressure was high the same dose would cause a fall. Working upon the intestine, Spadolini (59) was able to cause contraction or relaxation of the gut upon stimulating the splanchnic nerve by varying the rates and the strengths of excitation, weak currents causing inhibition, strong currents causing contraction. Gruber (60) was able to produce either vaso-dilatation or vaso-constriction with epinephrine in skeletal muscle vessels in which the tone of the vessel wall had been changed by cutting the nerve and his findings have been supported by Dale and Richards' (61) work on the capillaries in the same organ. Kolm and Pick (62) demonstrated the reversal of the action of epinephrine on the heart. Thomas and

Wheelon (63) were able to observe either relaxation or contraction of the antrum pylorus and duodenum and Carlson independently found similar effects on the same organs following the injection of epinephrine. In addition Carlson, Boyd and Pearcy (65), and Carlson and Litt (66) state that the splanchnics carry both motor and inhibitory fibres to the cardia of the cat and the resulting action at the time of stimulation is dependent upon the tonic state of the organ. M'Crea and M'Swiney and Brown and his co-workers noted similar changes (67) and Sharpy-Schafer (68) states that the sympathetics carry both inhibitory and motor nerve impulses.

Assuming therefore that there are two kinds of nerve impulses in the splanchnic trunk and the action is determined by the state of tone of the vessels, it is possible to explain all the changes on the kidney recorded in the literature. Schwartz (69) observed constriction of the kidney following cutting of the splanchnic while all other investigators noted an increase in volume.

Do the autonomic nerves contain secretory fibres for the kidneys? Since those who maintain the presence of such nerves have never proved satisfactorily that the increased secretion recorded could not be produced by the vaso-motor changes upon cutting the nerves only, this question can be answered in the negative more easily than in the affirmative. However, in justice to those who believe that the kidneys are supplied with secretory nerves it is only fair to present briefly their arguments.

Barcroft and Brodie (70) studied the gaseous metabolism of the kidney in dogs under normal conditions and during diuresis. They found the onset of diuresis not necessarily accompanied by an increase in the rate of flow of blood through the kidney and even when an increase was present it was never in proportion to the acceleration of the urine flow. Asher and his co-workers (71) are the strongest proponents for the theory of direct secretory nerve supply to the kidney. They believe the urine is normally diminished by the impulses over splanchnic nerves and increased by those of the vagus nerves. The increase in urine flow following the cutting of the splanchnic nerve they attribute to the loss of this inhibitory action and Asher (71) and Pearce (33) attempted to show that the vagus contained specific secretory nerve fibres to the kidney similar to the secretory nerve fibres in the chorda tympani to the submaxillary gland. They admit that the splanchnic nerve contains vaso-motor nerve fibres but they believe that it contains in addition to these inhibitory secretory nerve fibres. Stöhr (16) from his experiments concluded that there can be no doubt but that the entire vascular system

as well as the secretory system of the kidney is under the influence of nerve impulses. In dogs and cats Pearce (33) noted increased secretion of urine upon stimulation of the peripheral cut end of the vagus nerve without vaso-dilatation and naturally concluded that the vagus carries secretory nerves to the kidney. He, however, later openly denied these findings (72). Secretory nerve fibres in the vagus to the kidney were also claimed by Mauerhofer (73) and others but denied by Masius (28), Arthaud and Butte (24), and Schneider and Spiro (27). They obtained reduced secretion of urine by stimulation of the vagus, but they did not take into consideration the effect of changes in blood pressure thus produced on the kidney.

That ammonia may be formed in the kidney was demonstrated by Benedict and Nash (74) and confirmed later by Holmes and Patey (75). These findings gave greater impetus to research on secretory nerve fibres to kidneys than ever before. Immediately Ellinger (76) brought forth evidence supporting his view that the vagus and splanchnic nerves affect water excretion in the same way, but that of the solid constituents of the urine in the opposite way. Thus Ellinger and Hirt (77) divided the nerves to the kidney functionally into two classes: 1, those regulating the quantity, and 2, those regulating the composition of the urine. The lower splanchnic and the vagus nerves regulate the quantity; thus when the lesser splanchnic nerves are divided there is an increased excretion of water and salt, while the other constituents are only diluted. This change they believe results from increased blood flow through the kidney, so that what is practically glomerular filtrate appears in the ureters. Impulses accelerating diuresis they believe pass down the vagi. They also believe that certain nerves control the composition of urine without affecting the quantity, i.e., the greater splanchnic nerves carry impulses to the kidney causing increased synthesis of ammonia and increased excretion of uric acid and phosphates. Whereas the lower sympathetic fibres (Nervi renalis inferior) have the opposite effect, i.e., without influencing the excretion of water they inhibit the formation of ammonia and the excretion of uric acid and phosphates. The changes in the urine reported by Ellinger and Hirt (77) are, however, too insignificant and require critical confirmation before much importance can be placed upon them in proving secretory nerve fibres to the kidneys.

Hara (78) studied the secretion of urine in denervated and normal kidneys in a dog with two bladder fistulae. The acidity, freezing point, total urea and chloride excretion remained different in the two kidneys for weeks

and the concentration was less on the denervated side. This difference was greater on a meat than on a milk diet. The total amount of solids excreted in the denervated kidney was greater. The excretion of dye was slower on the denervated than on the normal side, reaching its maximum later and ceasing later. He believed his research demonstrated the presence of secretory nerves. He, however, took no cognizance of the fact that the ureter of the denervated kidney was twice the diameter of the normal, indicating in all probability some obstruction to the free flow of urine at the site of the uretero-vesical junction. The findings of Hara (78) have been confirmed by Kichikawa (79). He argues that the implantation of the kidney in some other region does not disprove the presence of secretory fibres in the organ and cites other experiments in other organs in which the sympathetic and parasympathetic nerves were cut and behaved in a normal manner. He admits however that the changes in secretion he recorded could be due to changes in circulation but prefers to explain them on the basis of the nerve impulse acting on the permeability of the kidney cells. These findings of Hara and Kichikawa are not in accord with those of Milliken and Karr (52) who noticed the dye appearing in the urine of the denervated kidney in $\frac{1}{2}$ to $1\frac{1}{2}$ minutes as compared to the normal in 3 to 4 minutes. They also find the denervated kidney just as efficient as the normal kidney in the removal of salts. Other investigators (44, 69) have presented similar data. However Grek (45) presented data showing that section of the splanchnic caused not only diuresis on that side but also increased secretion of sodium chloride when sodium chloride solution was administered in place of water.

Another interpretation based upon identically the same experimental results is presented by Kusakari (80). Upon cutting the vagi in some dogs and the splanchnics to the kidney in others he arrived at the conclusion that the vagi are without effect but the splanchnics promote reabsorption of fluid in the tubules. As far as can be determined this is only another interpretation of results that can be explained more easily in a number of other ways, especially by changes in the circulation of blood through the organ thus affected.

The effect of denervation of the kidney on the histology of the kidney itself gives no clue as to whether secretory nerve impulses are transmitted to this organ through the autonomic nervous system and in the narrow scope of this review the author can only refer the reader to some of the investigations on this subject (81 to 92 inclusive).

To demonstrate that the autonomic nervous system does not carry

secretory fibres to the kidney proper the experiments on the transplantation of kidneys may be cited briefly. In these the kidneys were excised and transposed to another region of the body thus cutting not only all the secretory nerve fibres, if such are present, but also all the vasomotor fibres. Carrel and Guthrie (93) were the first to demonstrate that an animal will survive such an operation for some time. Lobenhoffer (81) studied in a detailed way the function of the kidney after transposition. He severed the renal pedicle and transposed the kidney to the splenic vessels. As far as he could determine a transposed kidney was able to meet the ordinary demands of life. These results have been repeated and confirmed many times. Zaaijer (94) reports a dog living 6 years after its single kidney had been transposed to the iliac vessels, thereby removing it from any normal nerve supply. His findings have been confirmed by a large number of investigators (83, 91, 95, 96 to 99, etc.). Koennecke (83) concluded from his experiments on dogs that a kidney deprived of its efferent nerves produces a urine which appears normal in composition but is deficient in saline content and amount as compared with the urine from the other kidney. He assumes that a kidney deprived of its nerves, and acting purely automatically suffices to maintain life under every day conditions but fails when greater demands are made upon it. Thus he states, "when the composition of the body fluids demands a rapid restoration of physiologic values, by increased diuresis and elimination of salts, a kidney that has been cut off from nervous communication with the rest of the body cannot adapt itself to the increased demands." Boeminghaus (91) refutes these statements.

Marshall and Kolls (100) have been most painstaking in their investigation and conclude that the burden of proof still rests with those who would assign a specific secretory inhibitory action to the splanchnic nerve aside from the changes which it causes by being the chief vasomotor nerve to the kidney. Yagi and Kuroda (101) showed that when the quantity of the secreted urine is altered by artificially regulating the blood supply of the kidney the total amount of constitutents varies accordingly. Yoshimura (102) came to the same conclusion. He sectioned the renal nerve on one side and found the secretion of urine three times as much on the impaired side when compared with that of the intact side. The total amount and the percent of sodium chloride was considerably increased but when the quantity of urine was made equal by applying pressure to the renal artery of the impaired side, the constituents became equal, i.e., the total amount and percent of sodium chloride excreted in the urine was the same in the two kidneys. Further-

more, the findings of Marshall and Crane (103), White and Martin (104), Yoshimura (102), Cushny (6), Bayliss and Fee (50), Biedl (54), Howell (105), and many other support the view that secretory fibres to the kidneys for the secretion of urine do not exist and the marked effects obtained in the majority of purely physiological experiments are all explicable by the changes produced in the blood flow through the kidneys.

It is a well known fact that the formation of urine can be influenced by reflexes (51, 52, 83, 106–112, and others). These reflexes made known by either oliguria or anuria can be better and more readily explained on the basis of angio-spasm than upon the theory of inhibition of the secretory mechanism and present further evidence that the secretion of urine by the kidney is in all probablity dependent upon its vasomotor control rather than specific secretory nerves.

Drugs. To disprove or prove that an organ may be innervated with sympathetic or parasympathetic nerve fibres the actions of certain drugs upon these organs are frequently employed The drugs commonly considered as specific sympathico-mimetic are epinephrine and ergotoxin (1, 57, 113, 114), and those possessing parasympathico-mimetic actions are pilocarpine, acetyl-choline, and atropine. Although we realized that these are not infallible and there is considerable debate as to whether or not they act specifically, nevertheless they serve this purpose in the majority of cases. When epinephrine is administered in small doses increased secretion of urine is noted (115, 116, 117). Jonescu (118) has never observed a primary vaso-dilatation of the kidney vessels in rabbits and believes these vessels are more sensitive to epinephrine than other vessels. Ogawa (117) records very definite changes in the kidney vessels from epinephrine administration dependent upon the dose and the tone of the blood vessels in the kidney at the time of the injection. Small doses cause vaso-dilatation whereas large doses cause a decrease in the volume of the kidney. This dilatation he believes is a primary effect of epinephrine and the more dilute the solution the more quickly it occurs. He concludes that not only the vaso-constriction but also the vaso-dilatation of the kidney vessels is a direct action of epinephrine and the kidney vessels possess not only vaso-constrictor nerve endings but also vaso-dilator ones, and epinephrine acts upon the receptive substance in the end apparatus. Marshall and Davis (119) and Marshall and Kolls (100) observed polyuria following unilateral adrenalectomy in dogs. The diuresis occurred in the kidney that was on the same side from which the adrenal gland had been removed. These results are apparently the same as those of Ellinger and Rhode (46) after complete denervation.

According to Hirt (10, 64) this surgical procedure removed not only the adrenal gland but also most of the renal nerves on that side and consequently the results cannot be attributed to lack of adrenal secretion.

Kusakari (120) advanced the theory that epinephrine increases tubular reabsorption as well as decreasing the excretion by vaso-constriction of the glomerular vessels. Arnstein and Redlich (121) believe that the changes in the circulation in the kidney are not sufficient to account for the decreased excretion of urine following epinephrine and ergotoxin injection and therefore conclude that the anuria is due to a physico-chemical change in the cells plus the vasomotor change.

Another theory advanced by Biberfeld (116) is that epinephrine produces a hyperglycemia and a resultant glycosuria and the diuresis is due to this excess of sugar. A number of men have advanced the theory that renal diabetes is due to the vegetative nervous system in the kidney. Here again the reader is referred to some of the papers from the voluminous literature on the subject which can not be presented here (122–131). Kokkalis (132) noted that morphine caused glycosuria more readily in dogs with the kidneys innervated than in those with denervated kidneys and this difference he believed was due to the absence or presence of the vegetative nervous system in the kidney. He previously attributed this to a hyperfunction of this system. Teshendorf (133), Grunke (134), and Hetényi and Pogány (135) noted inhibition of phlorhizin diabetes after the injection of ergotoxin which paralyzes the motor sympathetics to the kidney. This is in harmony with the findings of Kemper (136) who produced an increased glycosuria by the injection of epinephrine. Hetényi and Patai (137) were unable to support Kemper's findings in human subjects.

The influence of the vegetative nervous system upon renal glycosuria was also studied by Goldberg (138). From her results she concluded that since phlorhizin glycosuria is due to renal disturbance and since ergotoxine and atropine are negative sympathetics and vago-tropic substances and inhibit the action of phlorhizin glycosuria, renal glycosuria from analogy therefore must be of a vegetative nervous system origin.

Dünner (139), Bertram (140), and Dünner and Mecklenburg (141) believe the parasympathetic nervous system responsible for this disturbance since it can be inhibited by atropine. Nakazawa (26) carefully studied the action of atropine and pilocarpine upon the kidney in situ in rabbits, but perfused with Ringer's solution, its nerves being intact with the central nervous system. In such animals small doses of pilocarpine hydrochloride injected intravenously had no influence either

upon the urinary secretion or upon the volume of the organ. If large doses were rapidly injected intravenously they produced a sudden decrease in volume and a simultaneous decrease in the secretion of urine. Thus, pilocarpine, when quickly injected, produced a transitory and prompt constriction of the renal vessels, with rapid recovery. The constriction, however, is of central origin since the drug could not reach the organ. When pilocarpine was injected into the perfusion fluid he observed not a constriction but a dilatation of the vessels in the kidney. He carefully determined whether the action of pilocarpine was due to paralysis of the muscular coat of the blood vessels, paralysis of the vaso-constrictor end apparatus or due to stimulation of the vaso-dilator end apparatus. Since epinephrine is still active after the administration of pilocarpine he infers that the action of pilocarpine is upon the vagal nerve endings in the kidney, which inference is supported by the observations of Ozaki (142) and Zamaguchi (143) who found the vaso-dilator action of cholin antagonized by atropine. However, actual experimentation showed Nakazawa that atropine has no influence upon the vaso-dilator action of pilocarpine and that the point of action of pilocarpine was upon the vaso-dilator end apparatus and not upon the vagus. This action of pilocarpine is not lost by degeneration of the vagal nerve endings in the kidney. Atropine alone has no effect upon the blood vessels or urinary secretion of the kidney. If, however, epinephrine is injected before the injection of atropine, then atropine is capable of causing vaso-dilatation in the organ and indeed it may depress the vaso-constrictor mechanism sufficiently to make the epinephrine almost inactive. These findings are supported by those of Nishimaru (144) who states that the vaso-constrictor nerve of the lung is excited by epinephrine and paralyzed by atropine. Epinephrine however, always caused decreased volume of the kidney even after atropine administration. The observations of Kusakari and Takeda (120) confirm these results. They also observed pilocarpine to produce dilatation of the kidney vessels but both atropine and epinephrine decreased the secretion in the toad kidney. This decrease they attributed to increased tubule reabsorption and vaso-constrictor action of these drugs on the glomerulus. Hamet (145) studied the action of acetyl-choline on kidney function and noted vaso-dilatation with increased excretion of urine.

To summarize briefly, then, the kidneys are innervated by parasympathetic and sympathetic nerve fibres. The function of the parasympathetic fibres has not been proved satisfactorily, either by physiological or by pharmacological investigations. The sympathetic system sends both

vaso-dilator and vaso-constrictor nerve fibres to the kidney. Vasomotor changes induced by the action of these fibres suffice to explain the secretion of urine without the assumption of specific secretory nerve fibres being present.

Musculus spiralis papillae. Only a few investigations have been made on the spiral muscle of the papilla (146). No one has undertaken to investigate its autonomic innervation. A few experiments have been performed showing that, in all probability, these smooth muscles are innervated autonomically. Epinephrine when applied to the bath increases the contractile force, the rate of contractions and the general tone of the muscle, whereas pilocarpine slows the contractions. Neither atropine nor ergotoxine were tested. It appears therefore that these smooth muscles may be innervated by para-sympathetic as well as by sympathetic nerve fibres.

Kidney capsule. As far as can be determined with any degree of accuracy, the kidney capsule is innervated by efferent vasomotor fibres from the sympathetic system (147–156, etc.). Smirnow (19) studied the innervation very carefully and in addition to these fibres he noted end-organs in the capsule and considered these a part of an afferent system of fibres. Stöhr (154) differentiated between nerves innervating blood vessels and nerves innervating the capsule which he called "Nervi proprii." Lehmann (155) studied the innervation of the kidney with special reference to the capsule nerves and what effect their loss has after decapsulation. He employed human, monkey, rat, and guinea-pig kidneys, and observed, contrary to other investigators, the fact that both the dorsal and ventral vagus branches pass to the solar ganglia which in his case was a fusion of the coeliac and superior mesenteric ganglia. All fibres from the splanchnic major also reached this complex. From here the fibres followed the arteria renalis and reached the kidney hilus where again a ganglion was noted (the renal ganglion) which was anastomosed by nerve fibres to the sympathetic aortic plexus. Also fibres from the splanchnic minor made up the renal nerve. At the kidney hilus the nerve splits up, some fibres pass with the blood vessels into the kidney proper, some go to the ureter and kidney pelvis, and others to the kidney capsule. In the capsule the nerve fibrils run parallel with the blood vessels and nerve bundles were observed in the capsule proper. Complete denervation of the kidney can be accomplished only by cutting the nerves to the capsule and the kidney proper.

Using a silver impregnation method of staining, Fisher (156) studied the nerve supply of the kidney. In addition to this he performed

physiological experiments upon dogs. He freed the renal capsule from the kidney leaving only that portion near the kidney hilus intact. In dogs, electric and mechanical excitation of this freed capsule of the kidney caused a gradual decrease in the volume of the kidney without affecting the blood pressure. He therefore reasoned that the nervi proprii of the kidney capsule were not connected with blood vessels, that they were not motor to smooth muscles in the capsule, but that they carry sensory afferent "sympathetic" nerve fibres which involve reflexly the vasomotor centre. Other investigators (157, 158, 159) have maintained sensory nerve fibres in the kidney capsule.

However, we do not believe these nerve fibres to be of the sympathetic system. They are of the visceral afferent system, but do not belong to the sympathetic division of the autonomic nervous system. It therefore appears that the capsule is innervated by vasomotor nerve fibres from the autonomic system, but its smooth muscle fibres are innervated by neither inhibitory nor motor nerve fibres directly.

THE URETER. The wall of the ureter is pinkish to bluish white in color. It consists of an external fibrous coat, a middle coat of plain muscular tissue, and a mucous lining.

From the physiological point of view the most interesting yet least understood question in the histology of the ureter, is the structure and distribution of the nervous elements in the ureteral wall. According to the literature it is richly supplied with nerves, which are derived from the plexus renalis, spermaticus (or ovarian) and the hypogastricus inferior (16, 160–165).

The fibres from the plexus renalis supply the hilus of the kidney and the upper half of the abdominal ureter, those from the plexus spermaticus innervate the lower half of the abdominal ureter, and those from the plexus hypogastricus the pelvic ureter. These nerve trunks send nerve fibres along the courses of the arteries in the ureter which form a rich network of nerve fibres in the adventitia known as the "Grund-plexus" by Engelmann (166). From this meshwork, nerve fibres pass into the tunica muscularis, throughout its length.

Some nerve fibres can be demonstrated to pass through the muscular coat into the mucosa (20, 167). Disselhorst (167) described both medullated and non-medullated nerve fibres.

Although Retzius (18) demonstrated end-organs in the smooth muscles of other tissues than the ureter and Lustig (168) believed he was able to demonstrate them in the musculature of the ureter, the consensus of opinion is that such a special end-apparatus does not exist in the ureter.

Engelmann (166) was unable to demonstrate them, and by calculation estimated one nerve fibre to every 25 or 50 muscle fibres. Neither Wolff (169), Häbler (170) nor Disselhorst (167) has been able to demonstrate to his satisfaction special nerve endings.

The presence or absence of ganglia and ganglionic cells in the various organs containing smooth muscle has been one of the most actively studied questions since the demonstration of ganglia by Remack in 1840. He found peripheral ganglion cells in several organs of the alimentary canal and the urinary tract. Many found ganglia in the ureter of the bird. A detailed investigation of this subject was first made by Engelmann (166) in 1869 on cat, dog, human, and rabbit ureters. According to this experimenter, a number of nerves enter the organ at two points, above at the renal hilus from the plexus renalis, and below, at the bladder end, from the sympathetic or vesical plexus. These nerve trunks build a plexus, the "Grund-plexus," with large and long meshes in the outer fibrous coat along the whole length of the ureter. Ganglion cells are absent in this plexus except in the lowermost 30 or 40 mm. and for an occasional small ganglion in the hilus renalis. The ganglia appear in the adventitia but in neither the muscular nor mucous layers. In the hilus renalis they are made up of 3 to 5 ganglion cells but in the adventitia near the bladder they may contain 100 or more cells. Because of these findings, Engelmann believed the contractions of the ureter to be of myogenic and not of neurogenic origin. His research gave impetus to a large number of researches upon the subjects both of presence or absence of ganglia and of the origin of spontaneous contractions of the ureter. Obersteiner (171) in 1871 confirmed Engelmann's findings.

In 1878 this subject was reinvestigated by Dogiel (172) using ureters obtained from the dog, pigeon, rat, and tortoise. He was able to demonstrate, to his own satisfaction, not only ganglia in the adventitia at the two extremes of the ureter, as Engelmann had previously shown, but ganglia throughout the adventitia of the entire ureter. Few cells were found in the middle third but in the lower third ganglia containing more than 200 cells were noted. Maier (15) also studied the nerve supply of the ureters of rabbits, pigs, calves, and humans. He was unable to demonstrate as large ganglia as were observed by Dogiel but noted smaller ganglia of from 8 to 10 cells. These he found in the adventitia and in the submucosa near the muscular layer. Occasional ganglion cells were found between the outer longitudinal and circular muscular layers, but in some instances these appeared to be only bipolar cells. With minor changes concerning the distribution and the number of cells

in each ganglion, Dogiel's and Maier's findings were supported by the observations of Protopopow (173), Alksne (174), Primbs (175), Satani (176), and Boeminghaus (177).

Investigating the ureter of man, pig, cat and dog, Hryntschak in 1925 was unable to demonstrate ganglia and ganglion cells in the kidney pelvis, and upper $\frac{2}{3}$ of the ureter. In the lower $\frac{1}{3}$ of the ureter and in the intramural ureter of the cat, ganglion cells as well as ganglia were demonstrated in the adventitia. These were absent in the pig ureters. Ganglion cells were not observed in the muscular and mucous layers. He has shown many ganglia on the outer surface of the adventitia but he questions the possibility of these being concerned with peristalsis.

It is well known that nicotine affects the ganglia of both the sympathetic and parasympathetic systems. Using the action of nicotine to determine the presence of ganglia both Macht (178) and Satani (176) believed they were able to demonstrate them throughout the ureter. This work has met with considerable criticism by Hryntschak (13) who believes that nicotine stimulates the smooth muscle directly as it stimulates striated muscle in chicken and frogs as observed by Langley (179), and Edmunds and Roth (180). Gasser (181) was unable to show contraction of smooth muscle of the intestine by the addition of nicotine to the bath when the segment was robbed of its ganglia. There is no doubt but that the autonomic nervous system innervates the ureter, but from this maze of contradictory evidence as to the presence of ganglion cells one can only conclude that both ganglia and ganglion cells do occur in the adventitia at the two ends of the ureter. Whether they are present in other parts of the organ is still doubtful, although there seems to be good evidence that such is the case.

Another complicating factor in the study of the neurogenic or myogenic origins of spontaneous rhythm in the ureters and the presence of autonomic nerves is the presence of interstitial or reticular cells. These cells have been demonstrated to exist in the ureters by Van Esveld (182). He like Lawrentjew (183), Tiegs (184), and many others, believes these cells to be a part of the nervous system, excitable by nicotine and other drugs and perhaps belonging to the autonomic nervous system. Van Esveld has shown that these cells can be impregnated with nerve cell stains as can the ganglion cells. He has also shown that "nerve cell free" preparations of smooth muscle can still be excited with nicotine contrary to the findings of Gasser (181). Other men are firm in their belief that the interstitial cells are of connective tissue origin (185).

The function of the ureter is to convey the urine from the kidney to the

bladder and in this capacity it is not simply a passive conductor but an active organ. The propulsion of urine is brought about mainly by peristaltic contraction (166, 13, 177, 186). In addition the ureter shows pendular movements as well as antiperistaltic contractions (166, 186).

As to the origin and the mechanism of propagation of the peristaltic contractions considerable differences of opinion have arisen (166, 187–194). Budge (195), and Valentin (4) believed them to originate from impulses sent from the central nervous system. Valentin noted in addition that it was possible to cause peristaltic contractions of the ureters by stimulation of the sympathetic nerve and by direct excitation of the muscle wall. Since these contractions are noted in excised muscle, (191, 178, 192, 196, 176, 13, 186), the origin of these must be independent of the central nervous system and whether the propagation of the impulse be myogenic or neurogenic is still a moot question. It should be remembered that the ureter is a highly specialized involuntary muscular organ, and Engelmann believed that in life smooth muscle is a syncytium, and that contractions are transmitted by molecular activity without the inter-relation of nerves.

Cessation of secretion by separating the kidney from all its proximal attachments according to Protopopow (173) slows the activity of the ureter temporarily, but tying the ureter at the kidney pelvis results in depression followed by complete inactivity. He believed this result to be due not only to the exclusion of urine but also to the blocking of the nerve supply to the ureter from above. It is not necessary, however, to assume such interference and his results can better be explained, according to Engelmann and Gruber, upon the basis of changing the position of the "pacemaker." Thus by tying the pelvis of the kidney, the more rapidly contracting portion is cut off and consequently the ureter contracts at a much slower rate. Protopopow, however, also demonstrated an anastomosis between the inferior mesenteric ganglion and the hypogastric plexus. These fibres, he states, are motor in origin. He found that cutting the splanchnic major nerve on one side produced a very marked slowing of ureteral peristalsis. Stimulation of the peripheral stump increased the rate of contractions providing the ureter was not ligated at the kidney pelvis. If the ureter was ligated at the renal pelvis before stimulation, no result of the stimulation was observed. Valentin (4) also noted splanchnic stimulation to accelerate ureteral peristalsis. This is supported by the observations of Langley and Anderson (197), who saw the main hypogastric nerve give off three ureteric branches and their results have been confirmed by Jackson (290). Caporale (198)

observed dilatation of the ureter after section of the sympathetics. That the lesser and inferior splanchnic nerves affect the kidney and ureter has been suggested by Latarjet and Bertrand (511).

Fagge (199) and Stern (191) observed an acceleration of the movements of the ureter upon stimulation of the hypogastric nerve. A similar action on the ureter upon stimulation of the hypogastric nerve trunk is described by Elliott (57). However, in the ferret's ureters, which are markedly active, he found no change in the movement upon hypogastric nerve stimulation, nor did epinephrine influence its contraction. Henderson (200) states that stimulation of the hypogastric nerve and splanchnic nerves will often produce waves in a ureter which seemed paralyzed from too much distention.

In studying the downward peristaltic waves in the fowl's ureter, Gibbs (201) was convinced that these movements appear to be controlled by the sympathetic nervous system and Hepburn (202) maintains that, in order to prevent growth and regeneration of the sympathetic nerves which are cut in denervation of the ureter, the ureter has to be displaced from its normal bed. Wharton and Hughson (203) maintain the sympathetic motor innervation, but deny sacral innervation.

Pharmacologically, the presence of both sympathetic and parasympathetic nerve endings in the ureter has been shown by Satani (176) and he was able to confirm this by stimulation of the splanchnic and pelvic nerves. He concluded, therefore, that there exist in the ureteral wall, sympathetic and parasympathetic nerve endings which are connected with the pre-ganglionic fibres of the thoracico-lumbar and sacral outflow respectively. These findings are supported by the previous findings of Stewart and Barber (204) who noted a decrease in the frequency of contractions upon stripping the ureter, but contradicted by the findings of Engelmann (166) and Hryntschak (13).

Almost all studies concerning the effects of drugs on the ureter have been directed toward the solution of the puzzling problem of innervation. If we adhere to the schema that epinephrine and ergotoxin are purely sympatho-mimetic drugs and pilocarpine and atropine parasympatho-mimetic drugs then the answer to this problem, pharmacologically, has been more satisfactory and clearer cut than the anatomical and physiological efforts. However, inasmuch as some doubt has arisen recently as to the exact points of action of these drugs these results no longer seem so conclusive and exact.

Elliott (57) applied epinephrine 1:2000 solution directly to the wall of the ureter in the intact animal and found it to produce flaccid paralysis.

He concluded that the ureter was not innervated by motor fibres from the sympathetic system, because neither stimulation of the nerve trunk nor the application of epinephrine caused contractions. He does not report the brand of epinephrine used nor does he state whether the drug applied to the ureter was acid, neutral or alkaline in reaction. Roth (196), Gruber (186), and Hofbauer (221) claim that solutions of epinephrine which have a pH of less than 7 cause relaxation of tissues as a rule. It may, therefore, be that the acidity of the epinephrine used by Elliott more than counteracted the action of epinephrine itself. Moreover, Gruber has demonstrated that "adrenalin chloride" and "suprarenalin" are highly acid in reaction.

Lucas (192), Brodie and Dixon (205), Henderson (200), Roth (196), Macht (178), Satani (176), and Gruber (186) have shown epinephrine to have an excitatory effect upon isolated segments and rings of ureter. Satani and Lucas made observations on the effect of epinephrine upon the ureter in situ. All of these observers agree that epinephrine increased the rate, tone, and in some cases the amplitude of ureteral contractions. Roth and Gruber observed no variations in the effect of epinephrine on different parts of the ureter, but Satani found the greatest change in the upper segment, the response decreasing progressively toward the bladder. In excised whole pig ureters Gruber was able to change the location of the origin of the contraction wave and reverse the direction of the peristaltic wave by the addition of epinephrine to the bath surrounding the less active part of the segment. In this manner he was able to produce either peristalsis or antiperistalsis in the same ureter. This investigator also observed the rate of the conduction of the contraction wave to be more rapid when epinephrine was added to the bath in which the ureter was contracting.

Both Macht (178) and Satani (176) demonstrated increased peristaltic activity in the ureteral rings and segments by the addition of ergotoxin to the bath. They found that if, following ergotoxin, epinephrine was added to the bath, the increase in tonus and in rate of contraction previously observed is no longer obtainable but in its place inhibition of the contractions and loss in tonus are noted. This is in keeping with the action of these drugs upon other organs sympathetically innervated, and Macht considers, correctly we believe, that this is further proof of the presence of sympathetic motor fibres, for ergotoxin inhibits the motor effect of epinephrine but does not interfere with its inhibitory effect. Here again we find sympathetic nerve trunks carrying two kinds of fibres, motor and inhibitory, to smooth muscle.

Pilocarpine has been used to study the presence of the parasympathetic nerve supply in the ureter. Lucas (192), Roth (196), Penfield (206), Macht (178), Satani (176), Pentimalli (207), and others, have used pilocarpine and agree that it increases ureteral activity although Lucas, Roth, and Penfield feel that this effect is only slight. Satani found the greatest stimulation of pilocarpine in the bladder segment, with a gradual diminution toward the kidney pelvis. From his results with epinephrine and pilocarpine he concluded that the innervation of the upper part of the ureter is mainly from the sympathetic and that of the bladder end mainly from the parasympathetic systems, a view also held by Young (163). The results with atropine were very variable. Macht (178) observed only relaxation and decreased activity, Satani (176) and Protopopow (173) observed increased activity with weak solutions and decreased activity with more concentrated ones, while Pentimalli reports only increased activity. Doubtful results were reported by Lucas, Roth, and Boulet (208).

Before we can say positively whether or not the ureters are innervated by both sympathetic and parasympathetic fibres many more experiments will have to be performed. It may be that pilocarpine acts in the ureters upon the sympathetic system the same as it supposedly does in the urinary bladder (209). If such is the case then the picture becomes more complicated than before. Until further evidence to the contrary is brought forth, we must assume therefore that the ureters are innervated by the autonomic nervous system, inhibitory and motor sympathetic fibres and motor parasympathetic nerve fibres as well.

THE URINARY BLADDER. In order to understand the autonomic innervation of the urinary bladder it is important first to understand the structure of the organ. As far as can be determined, it is composed of four layers, namely, serous, muscular, submucous and mucous coats. Of these, from the standpoint of autonomic innervation, the muscular coat is the most important. Authors disagree as to the arrangement of the muscular fibres. According to Da Costa (160) and Cunningham (210), it consists of an external longitudinal, a middle circular and an internal longitudinal layer. The middle circular layers are very thinly and irregularly scattered on the body of the organ and though to some extent placed transversely to the long axis of the bladder, they are for the most part arranged obliquely. These writers believe that, toward the lower part of the urinary bladder, around the neck and the commencement of the urethra the circular fibres are disposed in a thick circular layer, forming a vesical sphincter which is continuous with the muscular fibres of the

prostate gland in the male and with the muscular wall of the urethra in the female.

Griffiths (211) describes but two muscular layers in the bladder, an outer longitudinal and an inner circularly arranged. The longitudinal muscle bands, more frequently called the musculus detrusor urinae, take an oblique or transverse course around the lowest segment of the organ which can best be seen in the dog, but also seen in the cat. In mechanical obstruction the internal circular coat hypertrophies and becomes 3 to 4 times as thick as the external longitudinal muscle. Griffiths has been unable to find a sphincter at the neck of the bladder and has been unable to find a thickening of the muscle around the commencement of the urethra sufficient to constitute a sphincter or indeed anything approaching one. In fact according to this observer the arrangement of muscle is such that contraction of one set of fasciculi independently of another is impossible because the fasciculi run in their course from one plane or stratum into another, from longitudinal to oblique to transverse and vice versa. The triangular area between the entrance of the ureters and the urethra usually called the trigone requires special mention. Embryologically its origin differs from that of the fundus of the bladder in being of mesodermal origin while that of the fundus is of ectodermal origin (163, 212, 213) and physiologically it responds differently to autonomic nerve excitation from that of the body of the bladder (178, 163). According to Griffiths (211), it is a distinct structure only in man and monkey. This observation is not confirmed by Gruber (214) and other investigators (see table 1.)

In the search for the autonomic innervation of the bladder four methods have been employed by the various investigators. The first, in which physiological experiments were performed (215–217); 2, histological investigations (17, 18, 182, 189, 218–220); 3, anatomical observations (22, 222, 224, 226); and finally 4, a combination of these (227, 230, and others). From the large number of published experiments upon monkeys, dogs, cats, rabbits, ferrets, guinea pigs, and other mammals it is now absolutely certain that the autonomic innervation of the bladder comes from two sources, namely, the sympathetic formed by fibres from the thoraco-lumbar autonomic nervous system and the parasympathetic the origin of which is the sacral branch of the autonomic nervous system (57). Whether it also receives fibres from the vagi is to be doubted, as will be pointed out later.

The origin and route taken by the efferent nerves in reaching the bladder can be given only in a general way. They vary not only in their

origin and the pathway taken in the different species of animals, but also in different animals of the same species and from the right side to the left side in the same animal. The error commonly made in experimental work is the old one of arguing from the particular to the universal. In the realm of this report it is impossible to give all the ramifications and variations in either the hypogastric or pelvic (nervi erigentis) nerves.

TABLE 1

A table showing the response of the urinary bladder (circular and longitudinal muscle) and the trigone to hypogastric nerve and pelvic nerve stimulations

++ strong contraction, + contraction, +− contraction followed by relaxation, − relaxation only, 0 no effect.

AUTHOR	DATE	ANIMAL	HYPOGASTRIC NERVE			PELVIC NERVE (NERVUS ERIGENS)	
			Detrusor urinae	Sphincter	Trigone	Detrusor urinae	Sphincter vesicae
Budge	1864	Dog	+			+	
		Cat	+			+	
		Rabbit	+			+	
Sokownin	1874						
	1877	Dog	+	+		+	
Gaskell	1886	Dog	+				
Langley	1890	Rabbit	+				
	1891	Cat	+−		+−	+	+
Courtade and Guyon	1896	Dog	+	+		+	
Sherrington	1891	Monkey	+			+	
	1892	Cat	+				
Griffiths	1894	Cat	+ when relaxed − when contracted	+		+	+
Langley and Anderson	1895	Dog	+		++		
		Cat	+		++		
		Rabbit	+		++		
Nawrocki	1877	Cat	+			++	
Nawrocki and Skabitschewsky	1891	Cat	+			+	
Fagge	1902	Dog	+	+	++	++	0
Wlasow	1904	Dog	+			+	

TABLE 1—*Concluded*

AUTHOR	DATE	ANIMAL	HYPOGASTRIC NERVE			PELVIC NERVE (NERVUS ERIGENS)	
			Detrusor urinae	Sphincter	Trigone	Detrusor urinae	Sphincter vesicae
Elliott	1905	Ferret	++		++	++	
		Male goat	++		++		
		Indian Civet cat	++		++	++	
		Cat	+−	+	++	+	−
		Pig	+		++		
		Monkey	+		++		
		Dog	0		+	++	
		Rabbit	0			++	
		Female goat	0		+		
		Mongoose	0		+	++	
		Human	0		+		
		Guinea pig	Indifferent				
		Rat					
		Hedgehog					
Dale	1896	Cat	+−				
Barrington	1914	Guinea pig	++			+	
Fernsides	1917	Dog	+				
Jackson	1917	Dog	+−	+		++	−
Edmunds and Roth	1920	Cat	+−			+−	
Macdonald and M'Crea	1930	Cat	±			±	
		Dog	±*			±	
Nussbaum	1879	Dog				+	
Rehfisch	1900	Dog	0	+		+	0
Wolhynski	1930	Rabbit				+	
Schabadosch	1928					+	
Stohr	1928					+	
Sollmann	1923		Complex	+		+	
Denning	1926		−	+		+	
v. Zeissl	1894	Dog	−	+		+	−
Beattle	1930	Dog	−	+		+	−
Frankl-Hochwort Frohlich	1904	Dog	−	+		+	−
Longet	1891		+			+	+
Disse	1904	Dog		+		+	−
Wlasow						+	0
Fellner	1883		+			+	

* Response determined by the original state of tone of the muscle.

TABLE 2

A table showing the effects of stimulation of lumbar and sacral nerve roots upon the urinary bladder

++ strong contraction, + contraction.

NAME OF AUTHOR	YEAR	ANIMAL	LUMBAR							SACRAL				
			1	2	3	4	5	6	7	1	2	3	4*	5†
Langley and Anderson	1895	Dog		+	+	+				+	++	++	+	
		Cat		+	+	+	+			+	++	++		
		Rabbit		+	+	+	+				+	++	+	+
Nawrocki and Skabitschewsky	1891	Cat				+	+			+	+	+		
Elliott	1905	Ferret		+	+	+				++	+			
Sherrington	1891	Monkey	+	++	++	+	+			++	++	+		
	1892	Cat		+	+	+	+			+	++	+		
Courtade and Guyon	1892				+	+	+				+	+		
Langley	1890	Rabbit		+	+	+	+	+		+	+	++	++	
Wlasow	1904	Dog			++	++	++			+	+	+		
Young			+	+	+	+	+			+	+	+		
Stewart	1899	Cat			+	+	+				+	+		
Gianuzzi	1863	Dog			+	+						+	+	+
Bobin	1924	Rabbit									+	+	+	
Budge	1864	Dog								+	+	+		
	1877	Dog								+	+	+		
Gaskell	1886	Dog									+	+		
Nawrocki	1877									+	+	+		
Nussbaum	1879									+	+	+		
Langley	1891	Dog								+	+	+		
		Cat								+	+	+		
		Rabbit									+	+	+‡	
Rehfisch	1900									+	+	+		
Wolhynski	1930	Rabbit									+	+	+	
Schabadorch	1928									+	+	+		
Stohr	1928											+	+	
Beattle											+	+		
Griffiths	1895	Dog								+	++	+		
Fearnside	1918										+	+	+	
v. Zeissl	1892									+	+	+		
Mayer										+	+	+		
Jackson	1917			+	+	+	+							
Waldeyer		Human									+	+	+	

* First coccygeal in dog and cat.

† First coccygeal in rabbit.

‡ Rabbit 6 lumbar vertebrae instead of 7.

Broadly speaking, the sympathetic fibres forming the hypogastric nerve originate from the anterior roots of the 2nd, 3rd, 4th, and 5th lumbar segments, although occasionally fibres are contributed by the 1st and 6th lumbar nerves. See table 2. These segments send their fibres by way of the white rami communicantes, preganglionic fibres, some to end in the inferior mesenteric ganglion, but other axons may extend forward in the hypogastric nerve and terminate in the visceral plexus or in the walls of the bladder itself. In those cases in which pre-ganglionic fibres end in the inferior mesenteric ganglion post-ganglionic fibres continue as the hypogastric nerve to the vesical plexus and thence to the bladder musculature; in the case of pre-ganglionic neurones terminating in the peripheral plexus, their post-ganglionic cells are found here. The axons of the latter are very short and end also in the bladder musculature. This possible arrangement forms the basis for the autonomic axon reflex first described by Sokownin (231). As a rule, however, the majority of pre-ganglionic fibres terminate in the inferior mesenteric ganglion and the post-ganglionic fibres proceed to the bladder from this point after passing to the hypogastric plexus and the vesical plexus. As said before, the fibres of the sympathetic group are carried regularly in the 2nd, 3rd, 4th, and 5th lumbar roots, but the strands leading the impulses from the sympathetic chain to the inferior mesenteric ganglion vary much in position. Usually these are four in number on each side, but sometimes three and sometimes five are observed (232). Even though their origin from the sympathetic chain is also subject to some variation, they arise always in the region of the 3rd, 4th, and 5th lumbar segments. Langley and Anderson (229) occasionally found a few fibres for the bladder, leaving the sympathetic for the pelvic plexus as low down as the 1st sacral ganglion. In most cases, however, the fibres which cause contraction of the bladder after section of the hypogastric nerves and stimulation of the lumbar sympathetic or the lumbar spinal nerves run to the pelvic plexus from the aortic plexus or from about the region of the 6th lumbar ganglion. According to these investigators it is rare to obtain a contraction of the bladder by stimulation of the sympathetics at or below the 7th lumbar segment. The aortic branch described by them, however, has not been observed in rabbits by Wolhynski (233) who investigated it in over 50 animals.

Inferior mesenteric ganglia. Generally speaking, the inferior mesenteric ganglion in the cat consists of four ganglia, two above and two below the inferior mesenteric artery about ½ inch distant from the aorta, connected by strands which with the ganglia complete a chain around

the vessel; however, a single ganglion is often found in place of the two upper ganglia and in one cat Langley and Anderson (229, vol. xx) found the four ganglia fused into a continuous ring of nerve cells and fibres, through which the inferior mesenteric artery passed. In some instances the nerve filaments connecting the upper and lower pairs of ganglia may be long and slender, and the ganglia of the two sides as much as ½ inch apart. As said before, in nearly all instances four nerve strands run from the sympathetic chain on either side to these ganglia and occasionally there is a 5th strand. Occasionally four on one side and five on the other. These strands have been called rami efferentes of the ganglion and the nervi mesenterici and according to Langley and Anderson (229) neither name is appropriate on account of their homology with the splanchnic nerve. They therefore call them the inferior splanchnic nerves, but because of possible confusion they renamed them the spinal rami of the inferior mesenteric ganglion. This, however, represents its complexity but imperfectly since Krause (222) describes two ganglia in the rabbit and an accessory inferior mesenteric ganglion which is believed to be the spermatic or ovarian ganglion by Langley and Anderson (229). Nawrocki (227) describes four small ganglia in the cat and one large oval ganglion in the dog as making up the inferior mesenteric ganglion. Nikolsky (237), Sokownin (231), and Medowar (238) also describe a single inferior mesenteric ganglion whose size is dependent upon the size of the animal and in large animals it averages 2 cm. in diameter. Rehfisch (234) finds but a single ganglion in the rabbit, 2 or 3 in the cat and 4 or 5 in the dog.

In the rabbit, Langley and Anderson (229, vol. xx) find above the inferior mesenteric artery and close to the aorta, a conspicuous ganglion, long and narrow, which has been called by Frankenhäuser (22), Krause (222) and others the inferior mesenteric ganglion. This ganglion is joined by nerve bundles with a smaller ganglion a little below. The largest of these strands has usually been called the hypogastric nerve and considered as a single unpaired nerve. It is, in fact, according to Langley and Anderson, composed of two hypogastric nerves united into a common trunk. These may be easily separated. Each of these two ganglia consists of a pair of ganglia bound together in a common sheath, upper and lower inferior mesenteric ganglia.

Krause (222) mentions two ganglia in the male rabbit. He says the hypogastric nerve arises from the hypogastric ganglion. This may correspond to the superior mesenteric ganglion described by Langley and Anderson (229) or to a group of cells on the main aortic strand just above

its point of division described as a ganglion by Frankenhäuser (22). Neither Langley and Anderson, nor Schabadasch (235) were able to isolate Frankenhäuser's ganglion, whereas Wolhynski (233) noted it in 45 per cent of the rabbits studied. This ganglion he called, as did Krause previously, the "ganglion accessorium." He described it as oval in shape, varying in size with the age and the size of the rabbit. This ganglion is ventromedial to the aorta and 1 to 2 cm. below the inferior mesenteric artery. Pisemsky (236) describes a dense nervous plexus in the region where Frankenhäuser describes his ganglion. He says it is connected with the inferior mesenteric ganglion by two to four nerve trunks. Its function was not given.

Histo-anatomy. Since post-ganglionic fibres make up most of the hypogastric nerve trunk, it assumes a grayish cast (234) and according to Langley and Anderson (229), Griffiths (211), and others, the greater part of the hypogastric nerve consists of non-medullated nerve fibres.

Langley and Anderson (229, vol. xvii), using special staining technique, studied the relative number of medullated and non-medullated nerve fibres in the hypogastric nerve trunk. They found the greater bulk of the hypogastric nerve to consist of non-medullated fibres which usually run in broad bands in the nerve trunk. The number of non-medullated nerve fibres varies in different animals and usually varies in the two hypogastrics; the right they found usually the larger, the number of nerve fibres varying in the left from 396 to 738 and the right from 566 to 918, while in the two hypogastrics together the medullated fibres made up 950 to 1650 of the total number of fibers present in these nerves. These fibres they found to be chiefly very small, more than 99.9 per cent having a diameter of 1.3 to 4μ and a considerable number of these very small fibres with a diameter of 1.3 to 1.5μ. They believe the sacral nerves send a variable number of fibres, up to two dozen, into the lowest end of the hypogastric nerve, but nearly all of these turn back with the hypogastric nerve fibres to the bladder and other visceral organs (229). A few small fibres, 1 to 5 sometimes, continue on in the hypogastric nerve. The presence of such fibres is indeed doubtful since Schabadasch (235) was unable to find such nerve strands. No medullated fibres were noted descending the sympathetic by way of the sacral rami (229) passing to the nervus erigens or the hypogastric.

Stewart (232) was unable to demonstrate nerve fibres running to the bladder in the sympathetic chain other than those in the hypogastric nerves although such fibres have been described by Gianuzzi and Nussbaum (240). However, Sokownin (231) and Nawrocki and Shabitschew-

sky (227) failed to demonstrate them and Langley and Anderson's (229) results were variable. After leaving the inferior mesenteric ganglion the hypogastric nerve commonly divides about ½ to 1 inch from the ganglion, into two strands; the smaller and more dorsal Langley and Anderson called the accessory hypogastric. According to these experimenters, when this division occurs, as it sometimes does, very close to the inferior mesenteric ganglion, it is easy to determine that the larger strand, the hypogastric proper supplies chiefly the bladder and uterus or vas deferens, and that the accessory hypogastric supplies chiefly the rectum and and internal anal sphincter. In two animals stimulation of the accessory hypogastric nerve had no effect upon the bladder. The presence of this nerve has been noted by other observers (232).

Hypogastric nerve. Although it is generally admitted that the hypogastric nerves innervate the bladder, nevertheless the exact area in the organ supplied by these nerves and the responses caused upon their stimulation are still uncertain. Some men believe the hypogastric nerves supply only inhibitory fibres to the body of the bladder and motor fibres to the sphincter and trigone, others believe that they supply motor fibres to the entire organ. See table 1.

Elliott (57) in 1905 described three types of reaction upon hypogastric nerve stimulation, the results obtained depending upon the animal used. In the ferret, civet cat, and male goat it caused primarily contraction of the whole bladder; in the cat, pig and monkey, a slight constriction of the trigone without shortening of the urethra and at the same time relaxation of the rest of the bladder; in the dog, rabbit, female goat, and mongoose, only the trigone was stimulated. Indifferent responses were obtained in the bladder of the guinea pig, rat, and hedgehog.

In the two cats Elliott (57) was unable to get any response from the bladder upon stimulation of the hypogastric nerves, but in one animal he did observe contraction of the vas deferens, and in the other contraction of the female urethra. Upon further examination, it was revealed that the innervation of the bladder was entirely pelvic and that the hypogastric nerves took no part in its control. Moreover, in these animals epinephrine did not produce its characteristic response. The indifferent response of the guinea pig's bladder observed by Elliott was not confirmed by Barrington (241, p. 70) who reported a powerful contraction of the organ upon hypogastric nerve stimulation.

In the cat Langley (228), Dale (242), Elliott (57), Edmunds and Roth (209), and Sherrington (243) have shown that stimulation of the hypogastric nerve causes a temporary contraction of the bladder which

is followed in all cases by marked relaxation. The contraction produced is usually not sufficient to cause any micturition, but if it is strong enough it causes a discharge of only a few cubic centimeters of urine (57, 243). Sollmann (244) in his excellent article, dismisses the action of the hypogastric nerve upon the bladder with the term "complex."

Upon thorough study of the subject it is evident that the action of the hypogastric nerve is not as complicated as was formerly thought. There can be no question but that both the hypogastric and pelvic nerves carry efferent motor fibres to the bladder and their actions do not differ except in degree (229). There can also be little doubt that the hypogastric nerve carries both efferent inhibitory as well as efferent motor fibres to this organ.

The ultimate response of the bladder to hypogastric nerve stimulation no doubt is dependent upon a number of factors of which the most influential are the state of tonus present in the organ at the time of stimulation and the degree of irritability of the organ. In an experiment the depth and kind of anesthesia used affect the results obtained (217). Griffiths (211) noted the fact that if the tone of the bladder is high, stimulation of the nerve produced a small initial contraction which was quickly followed by a rapid and pronounced relaxation, which continued until the walls became flaccid. According to him this can best be shown by stimulating the pelvic nerve first thus increasing the general tone of the organ. Langley and Anderson's (229) findings are not exactly in accord with those just described. They observed that if the bladder was in a high state of tone, due to drying, hypogastric nerve stimulation had no effect. Moreover, they also presented evidence which showed that hypogastric nerve stimulation had no influence either upon the contraction or the rate of relaxation of the bladder from stimulation of the pelvic nerves. Elliott (57) believed contraction of the bladder was more readily obtained in animals upon excitation of the hypogastric when the pelvic nerves were sectioned before the stimulation.

Cats which were under deep chlorbutanol anesthesia were used by Edmunds and Roth. Their results can now be easily interpreted in the light of the excellent work of Macdonald and M'Crea (217). Because of its importance this work warrants presentation in considerable detail. They have demonstrated that the depth of anesthesia influences the response. In cats under deep anesthesia they always obtained relaxation of the organ but when the ether anesthesia became light, contraction predominated. The same findings were recorded when urethane and sodium luminal were used as general anesthetics.

In chloralosed animals (cats and dogs) the hypogastric nerves produced considerable excitation of the bladder which was not limited to the trigone region. In an ether-chloralosed cat the inhibitory effect changed to a contractor effect as the ether disappeared and again became an inhibitory effect as the general ether anesthesia was deepened. This contraction was not confined to the trigonal area but was a general well marked homolateral response. In decerebrated and spinal animals the effect of hypogastric nerve stimulation upon the bladder changed from relaxation to contraction as the ether wore off and vice versa. These findings are in accord with those upon other organs reported earlier in this paper (p. 502).

It is a well known fact that ergotoxin throws the motor fibres of the entire sympathetic system out of function (242, 57 and others). In those animals in which a normal contraction of the bladder occurs upon hypogastric nerve stimulation, such stimulation subsequent to the administration of ergotoxin is always followed by relaxation of the organ.

Macdonald and M'Crea (217) also noted the fact that ergotoxin reverses the reaction to hypogastric nerve stimulation if the normal response is contraction of the bladder. They say that ergotoxin increases the tonus of the bladder and they believe, therefore, that the motor sympathetics are fully stimulated rather than paralyzed. They interpret their results as showing that if in a mixed nerve the motor fibres are fully stimulated, only the inhibitory fibres remain to be excited and consequently relaxation of the organ occurs. They therefore conclude that in cats and dogs, the sympathetic and para-sympathetic nerves are both excitatory and inhibitory in the bladder. The effect, as stated before, depends upon two factors: muscle tonus and nature and degree of anesthesia.

In those animals in which contraction is produced upon stimulation of the peripheral end of the hypogastric nerve, the response is identically the same as that seen before stimulation of the peripheral end of the divided pelvic nerve. Like it, its effect is limited to the corresponding half of the organ and it affects equally all muscular layers of the organ (234, 211, 217).

Sacral autonomic nerve. It is generally agreed that the pelvic nerve is the main motor nerve of the bladder. However, unless one is familiar with the nomenclature applied to the sacral nerves supplying the urinary bladder one can be readily led astray. These nerve fibres are spoken of as the nervi erigentes (Eckhard, 246), as the pelvic splanchnic (Gaskell, 245), as the pelvic nerves (Langley, 1) and merely as the sacral branch

of the autonomic nervous system. The arrangement of the branches of the pelvic nerve varies in individual animals of the same species so that no general description can be accurate. See table 2. It is a small white nerve which arises usually by three roots, one large and the other two small, the large strand being supplied by the 2nd sacral nerve and the smaller strand or strands by the first and third sacral nerves. These roots join on their respective sides and form moderate size nerve trunks which in their course to the bladder are joined by the hypogastric nerves forming the hypogastric plexus with its ganglia. Due to the fact that far the greater part of this plexus consists of fibres from the pelvic nerve Langley and Anderson (229) believe it absurdly named the hypogastric plexus and prefer the term pelvic plexus. Some authors speak of the hemorrhoidal plexus (Frankenhäuser (22), Krause (222), Young (163)) and some others use these terms interchangeably. Valanti (508) describes a union of the plexus vesicalis with the plexus hemorrhoidalis medius, vesico-deferentialis, prostaticus or utero-vaginalis. From here the fibres pass to the vesical plexus and thence into the muscle layers proper. On this point authors are again disagreed. Some apparently describe the pelvic plexus as being the same as the vesical and others omit the vesical plexus completely. Pre-ganglionic fibres pass from the spinal cord, which in some cases terminate in ganglia in the pelvic plexus (234, 211) and in other cases end in ganglia in the vesical plexus on the bladder wall. In the former case the post-ganglionic fibres are long and in the latter case short. It is generally believed that the pelvic nerve arises at the 1st, 2nd, and 3rd sacral roots but even then a difference of opinion exists not only as to the origin of these fibres in the animals of different species but also in animals of the same species. See table 2. According to Wolhynski (233), the pelvic nerve may vary in origin on the two sides as well as vary in the total number of roots. Schabadasch (235) after studying the origin in 50 rabbits, reported the fact that he never noted the pelvic nerve to originate in a single animal from all three sacral roots and that the right and left pelvic nerves can originate from different spinal segments in the same animal. Thus in rabbits the nerve may originate from the 2nd sacral only or from the 2nd and 3rd, or 1st and 2nd, but never from the 1st, 2nd, and 3rd sacral spinal roots.

An additional factor which probably plays an important rôle in the great variation in origin of these nerve fibres is the arrangement of the pudental and sciatic plexuses, i.e., if these are posteriorly placed the origin of the pelvic nerve fibres is different from its origin in animals in which the other nerves are anteriorly placed even in the same species of animal (229,

vol. xvi). The abnormal number of thoracic and lumbar vertebrae also add confusion to this already difficult problem. Langley and Anderson (229, vol. xx) found in two dogs and one cat 6 free sub-thoracic nerves and in the rabbit 7 free sub-thoracic nerves. In the rabbit Sherrington (243) describes 6 lumbar vertebrae, but 7 in the cat and dog. Other abnormalities noted by Langley and Anderson in 3 cats and 5 rabbits were 7 free subthoracic vertebrae, 7 cervical vertebrae and 1 extra rib bearing vertebra making a total of 14 thoracic vertebrae in the cat and 13 thoracic in the rabbit. All of these variations add to the difficulty of the interpretation of the origin of these nerves. Griffiths (211) finds the trunk of the pelvic splanchnic nerve consisting almost entirely of small medullated nerve fibres measuring 2 to 3μ diameter, but there are also present, he says, a few medullated fibres of about 7μ in diameter. These small fibres lose their medullary sheaths in the ganglia.

In the cat Langley and Anderson (229) found normally about 3500 medullated fibres in the pelvic nerve (nervus erigens). Of these 1 to 2 dozen were 7μ to 12μ in diameter, several hundred were 4μ to 5μ in diameter and nearly all the rest were small nerve fibres.

From the hypogastric plexus the vesical branch of the pelvic nerve sends three sets of fibres to the bladder (234, 211). These are named according to their location the upper, middle and lower sets. These supply respectively the fundus, the middle, and the neck region of the bladder and the contiguous or first portion of the urethra. All of these are non-medullated nerve fibres, the medulla of the small fibres of the pelvic nerve being lost in the numerous ganglia on the fibres of the plexus. In this way Griffiths believes each lateral half of the bladder may be mapped out according to its nerve supply into three regions. Just as there are anatomical limitations, he says, so there are physiological limitations. Stimulation of the peripheral end of one of these branches after division induces contraction only in the area to which it is distributed, the contraction remaining limited and not spreading by virtue of continuity of the muscle fibres.

With but minor differences all workers agree that each pelvic nerve innervates but one-half of the bladder. Elliott (57), Sherrington (243), Griffiths (211), Langley and Anderson (229), Rehfisch (234), and Macdonald and M'Crea (217) noted strong contraction of the homolateral half and a weak contraction of the contralateral half of the bladder in the animal studied.

Frog. At most only a brief résumé of the autonomic innervation of the frog bladder can be presented here. Upon studying the published

literature on the subject, one finds some uncertainty as to its exact innervation. In 1886 Gaskell (251) showed that the hypogastric nerves caused contraction of the frog's bladder in the same manner as in the dog. The 8th and 9th nerve fibres passing by the homologue of the pelvic nerve (nervus erigens) of the mammal always caused contractions of this organ. Bernheim (247) in 1892 confirmed Gaskell in his findings. He noted contraction of the bladder on stimulation of the nerve running to it from the sciatic plexus. Anatomically he found that the nerve arose usually from the 8th and 9th spinal nerves but occasionally fibres also arose from the 10th. Waters (253) found variable effects upon stimulation of the 8th nerve.

Steinach and Wiener (248) observed that the bladder in the frog received motor nerves in almost equal proportions from the posterior and anterior roots of the 6th to the 9th spinal nerves, but the 7th, 8th and 9th most commonly, when stimulated, caused contraction of the bladder. An opinion slightly different was expressed by Horton-Smith (249). Motor nerves to the bladder and rectum of the frog were found in only the anterior roots from the 7th, 9th and 10th nerves. The sympathetic and sacral 8th was never found to contain sympathetic fibres for the frog's bladder, a fact confirmed by Dale (250). This latter investigator noted a gap between the 7th and 9th anterior roots and observed the 8th to contain no sympathetic fibres. These results were not supported in full by Langley and Orbeli (252), who say that the sympathetic nerves supplying the bladder have vaso-motor as well as viscero-motor fibres. Occasionally the 6th, but in nearly all cases the 7th, when stimulated, caused contraction of the bladder. They found the 8th usually ineffective. But the fibers from the 9th and 10th anterior roots forming pelvic nerves always caused contraction of the bladder.

Vagus nerve. There can be little doubt in the minds of most investigators as to whether or not the vagi innervate the urinary bladder. Inasmuch as the organ receives its principal motor supply from the pelvic nerves (nervus erigens) and some motor and inhibitory fibres from the hypogastric nerves, there is little reason for thinking that a third source of nerve control is necessary. According to Oehl (254) and Palumordwinov (255), the vagi contribute motor nerve impulses directly to the bladder. If these nerves are sectioned in rabbits and dogs and the peripheral end stimulated, they observed contraction of the organ. They also noted contraction of the bladder upon excitation of the central cut end of the vagus nerve. This they believe to be a reflex over the hypogastric and pelvic nerves. Their findings have not been confirmed either

by Kehrer (256) using dogs and rabbits, or by Bert (257), Nussbaum (240), Nawrocki and Skabitschewsky (227). As far as any conclusive evidence shows, the negative findings so outweigh the positive findings that the question of vagal innervation of the bladder can be wholly disregarded.

Ganglion. The presence of ganglia in the bladder wall is a common finding. Remak (258), Darwin (259), Obersteiner (171), Disselhorst (167), Maier (15), Disse (20), Bobin (226), Hryntschak (13), Michaelow (220), Stöhr (16), Müller (261), and Engelmann (166) described ganglia in the mammalian bladder, and Grünstein (262), Wolff (263), Lawdowsky (264), and Serebrjakow (265) in the frog, but they are not in accord as to whether they appear in all layers or only in certain strata of the bladder wall. They are, however, agreed that the region near the entrance of the ureters has the richest supply of these cells. Müller (261) described intra- and extra-mural ganglia and occasional ganglia in the submucosa. Michailow (220) observed ganglion cells in the mucous and submucous layers as well as in other parts of the bladder wall of the horse. However, Bobin and Hryntschak were unable to identify ganglia in the mucosa but admit the presence of nerve cells in the adventitia and intra-muscularis. Stöhr confirmed the findings of Hryntschak and Müller and presented evidence supporting the view that large numbers of ganglion cells are present in the bladder of the newborn human which vary in size from 10 to 100 cells each. He had noted them previously in the adventitia and he believed that they extended fairly deeply into the muscular coat. He describes five types of ganglion cells.

Using the nicotine and degeneration methods, Langley and Anderson (229, vol. xix) showed that the ganglia for the pelvic vesical nerves are on the wall of the bladder itself which fact has been confirmed by Elliot (57), Stewart (232), and Edmunds and Roth (209). That some few trophic centres for the hypogastric nerves may be located in the bladder wall cannot be denied.

Nerve endings. Upon reaching the bladder wall the post-ganglionic nerve fibres continue into the bladder proper as nerve bundles. The pre-ganglionic fibres end in the ganglia in the bladder wall and from here post-ganglionic fibres travel short distances as fibre groups (13, 16, 20). These nerve bundles are abundant in the adventitia (16), where they give off fibrils to the muscle and submucosa. In the muscle they are less abundant than in the adventitia and in the submucosa these are still fewer in number (16). These nerve fibres have been described under the names of nerve plexus, secondary plexus, intermediary plexus

intra-muscular network and end plexus. They divide into the finest terminal network and in this way supply the smooth muscle cells. The ultimate ending of these fibres, whether in or on the muscle cells, has been a point of much dispute. Two distinct views exist; Arnold (266), Frankenhäuser (22), Lustig (267), and Obregia (268) believe that the nerve fibre terminates in the involuntary muscle cell or often after passing through one cell it re-enters the intramuscular plexus passing to another cell before reaching its termination. Some of these investigators go so far as to insist that these fibres terminate in the nucleus of the cell itself (22, 266–268).

Other writers (17, 172, 219, 265, 269) are more conservative, and state that the fibrillae ultimately terminate on the outside of the muscle cell. Huber (270) was unable to demonstrate the terminal nerve endings of these fibres in the smooth muscle of blood vessels. These nerve fibrils were traced by Boecke (223) into the muscle cell and he believes that they form a reticular network about the nucleus of the cells. Stöhr (260) also believes that these nerve fibrils form a reticular network within the muscle cell and terminate as bare nerve endings with no special terminal structure. He admits, however, that the majority of muscle cells do not appear to receive such nerve fibrils. Another point of contention is the question as to whether or not all smooth muscle cells in the bladder receive nerve endings. According to v. Csiky (271) every muscle cell receives from 2 to 4 nerve endings. Gscheidlen (219), Lustig (267), Obregia (268), and Agababow (272) maintain that each muscle cell is provided with a nerve fibre. Fortunately such a view is not held by many investigators. Equally competent observers such as Disselhorst (167), Wolff (263), Köllicker (17), Huber (270), and Stöhr (16) do not believe that all muscle cells are provided with nerve fibres. Moreover, Langley (228) believes there exists approximately one nerve fibre for every 100 muscle cells and Engelmann (166) thinks there is one nerve fibre for each 25 or 50 muscle cells. With the better stain and the improved technique of the later investigators it can be stated with considerable assurance that the nerve fibres do not penetrate the nucleus of the cell and that not every smooth muscle cell is provided with nerve fibrils.

Muscular contraction. Whether or not the muscles of the bladder can contract without receiving impulses from the central nervous system has been discussed pro and con for some time. Morgan (274) and Schultz (275) believed the spontaneous movements to be purely of nerve cell origin. On the other hand Engelmann (166), Sertoli (277), Botazzi

(278), Straub (279), Stewart (280), Sherrington (243), and many others have shown that connection with the central nervous system is not necessary. Although Sherrington cut all the dorsal and ventral roots and the spinal cord at the level of the 12th thoracic vertebra in both cat and monkey, the contraction continued. Sections taken from the bladder wall showed spontaneous rhythmical responses. In all these experiments there is still the possibility that ganglia may be present in the muscle itself and that these cells may have an influence upon the spontaneous contractions of the organ. Certainly it has not yet been proven that they do not exert such an influence although most writers believe the spontaneous rhythmical contractions of the bladder are properties of the muscle cell itself.

Sensory nerves. While motor nerves may be explored by methods of direct stimulation with comparative ease, the demonstration of sensory fibres for the bladder is a matter of extreme difficulty due to the fact that the nerves stimulated may originate in the intestine or external genitals and not in the bladder proper. Apparently stimulation of any sensory nerve will cause contraction of the bladder (227, 280, 282). In his earlier work, Budge (215) reported that the hypogastric nerve trunk carried both motor and sensory fibres but later he thought that he found only sensory fibres. Gianuzzi (239) reports similar findings. It is generally agreed now that both the hypogastric and the pelvic nerves (nervus erigens) are mixed nerves. In the hypogastric nerve, according to Langley and Anderson (229), there is one afferent fibre to every 10 efferent fibres. Afferent fibres in both nerves have been described by a host of men (211, 215, 227, 229, 234, 235, 239, 243, 280–284, etc.).

It should be remembered, however, that these afferent fibres are not a part of the autonomic nervous system, although frequently classed as such by men using a loose nomenclature (225). They are probably visceral afferent fibres originating in the abdomen, but not necessarily in the bladder itself. According to Beattle (282) and Fearnsides (283) those in the pelvic nerves enter the cord by way of the dorsal roots of the 2nd and 3rd and possibly the 4th sacral spinal nerves (1st included 240) and those in the hypogastric nerve through the dorsal roots of the 11th, 12th thoracic and 1st and 2nd lumbar. Head and Riddock (284) noted the fact that cutting the posterior sacral nerve roots in man did not abolish sensations in the bladder.

Sensory end organs have been described in the bladder wall itself and Huber (270) classes these, erroneously we believe, as sensory end organs of the sympathetic system. Stöhr (16) described such special end organs

of the Pacinian body or corpuscle type and believed they represent the receptive endings of pain fibres. Michailow (220) and Grünstein (262) also described sensory nerve endings in the bladder wall, and Langley and Anderson (229) apparently believed the afferent nerves carry the sensation of pain principally.

Spinal cord centre. At this point it may be well to digress a little to mention the fact that many men believe that a reflex centre exists in the spinal cord which controls the tone and contraction of the bladder. Such an impulse would necessarily be carried by the autonomic nerves supplying the organ. In 1841, Valentin (5) stimulated the exposed spinal cord and brain (crura cerebelli, medulla oblongata, cerebral hemispheres and the floor of the 4th ventricle) of a rabbit and observed contraction of the bladder. These findings were supported by those of Budge (195) in 1864 and others who stimulated the cerebral peduncle and corpora restiformi and calamus scriptorium in dogs, rabbits and cats, and by Nussbaum (240), Nawrocki and Skabitschewsky (227) who stimulated the spinal cord. Bert (257), Mosso and Pellicani (281), and Nawrocki and Skabitschewsky (227) noted reflex contraction of the bladder involving the autonomic nervous system when the sciatic, median, inferior orbital, crural and other mixed nerves were cut and excited centrally.

In the cord these impulses travel in the lateral columns. They could not determine by hemisection of the cord at different levels whether or not crossing took place in short efferent neurones (281, 285, 286) to reach the bladder. According to Nawrocki, this reflex is mediated through the hypogastric nerve fibres and not through the pelvic nerves since cutting the hypogastric nerves abolishes the reflex whereas section of the pelvic nerves had no influence. Spiegel and MacPherson (286) stimulated the hypothalamus and observed contraction of the bladder. They too failed to determine by means of hemisection of the cord at different levels whether or not crossing took place in short efferent neurones.

A reflex centre has been described in the spinal cord as being involved especially in micturition but also as concerned with the reflexes from other sources than from the bladder back to the bladder itself. It is generally agreed that this centre lies somewhere between the 2nd and the 7th lumbar segments, the position varying with the species of animal and also with different animals of the same species. Masius (287) located it at the 5th lumbar in dogs and the 6th and 7th lumbar in rabbits. His findings were confirmed by Ott (285), Gianuzzi (239) and Kupressow

(216). Budge (195), Sokownin (231), Nussbaum (240), Goltz (288), and others describe it as being in the neighborhood of the 3rd and 4th lumbar segments. Stewart (232), Nawrocki and Skabitschewsky (227) place the upper limit of the centre at the 2nd lumbar segment and the lower limit in the 5th lumbar segment.

As an illustration of the involvement of this centre, Griffiths' experiments are described. He has shown that stimulation of the central end of one cut hypogastric nerve, all other nerves being intact, produces a reflex contraction of the bladder. If the pelvic nerves are cut, the reflex is abolished. On the other hand, if the pelvic nerves are left intact and the opposite hypogastric is cut, the reflex is not abolished. The reflex, he says, is therefore indirect through the lumbo-sacral spinal cord to the pelvic nerve. He also suggests that the reflex centre in the spinal cord is bilateral, each half controlling, as does each nerve, the corresponding half of the bladder. The two halves are connected by commissural fibres so that under ordinary circumstances they act in harmony with one another and are influenced equally by stimuli transmitted to one or the other, a condition similar to that found in the respiratory centre.

Hypogastric nerve axon reflex. In 1877, Sokownin (231) first pointed out the possibility of the inferior mesenteric ganglion acting as a reflex centre. In curarized cats he noted that stimulation of the central end of one hypogastric nerve, after the rami which run from the upper lumbar sympathetic to the inferior mesenteric ganglia had been cut, would still cause contraction of the bladder. If, however, the remaining hypogastric nerve was severed and the central end of the first again stimulated, no response resulted in the bladder. His findings were confirmed by Nussbaum (240), Landois (289), and by Nawrocki and Skabitschewsky (227). These investigators concluded, therefore, that the inferior mesenteric ganglion acts as a reflex centre and is under the influence of sensory fibres passing to it from the bladder itself. Langley (228), Langley and Anderson (229, vol. xvi), Elliott (57), Stewart (232), Jackson (290), Denning (230), and Griffiths (211) have placed an entirely different interpretation upon this same experiment. Langley and Anderson (229, vol. xiv) state that this experiment does not warrant the conclusion to which Sokownin comes, because the result might be explained in several other ways, namely: if the nerve fibres of the rami of one side divided and sent a branch to each hypogastric nerve; if some nerve cells of the ganglion sent motor fibres to each hypogastric nerve; if some of the ascending fibres of the pelvic nerve (nervus erigens) ran back in the opposite hypogastric nerve; if some fibres from the upper lumbar nerves

joined the hypogastric nerve below the ganglion and ran upwards and if the action current set up in one hypogastric nerve were strong enough to stimulate the neighboring fibres.

In the cat, other reflexes besides the contralateral contraction of the bladder are produced. These are: unilateral pallor of the uterus or vas deferens and prostate vesicle; slight contraction and pallor of the vagina or penis and some paling of the bladder itself (229). In dogs Langley and Anderson noted these reflexes to be slight and inconstant, but in rabbits always present and on the opposite side of the body from that of the nerve stimulated.

All of these actions they observed to be temporarily in abeyance after the injection of 10 to 20 mgm. of nicotine into a vein. In such an experiment the impulses set up in the central end of the hypogastric nerve were blocked in the inferior mesenteric ganglion, for nicotine did not annul the effects of stimulating the peripheral ends of the hypogastric nerve. Thus impulses passing up one hypogastric nerve traverse nerve cells in the inferior mesenteric ganglion, before they can reach the opposite hypogastric nerve. Had they made no further investigation, Langley and Anderson might have fallen into the same trap as the previous investigators for such findings certainly support the idea that a true reflex is present and the reflex centre must be in the inferior mesenteric ganglion. However, assuming that each nerve fibre had one and only one trophic centre or nerve cell, Langley and Anderson cut one hypogastric nerve and allowed time for degeneration to occur, after which central stimulation of the cut end of the hypogastric nerve still caused normal reflexes to occur. These reflexes therefore could not be due to fibres proceeding from a nerve cell on the peripheral course of the hypogastric nerve. If, however, the spinal branches to the inferior mesenteric ganglia were cut and time allowed for degeneration, stimulation of the central cut end of one hypogastric nerve gave no reflexes but stimulation of peripheral ends of the hypogastric nerve continued to produce the customary effects. These experimenters therefore concluded that the normal reflexes could not be due to nerve cells in the ganglion sending out two sets of fibres, one passing down each hypogastric nerve. Furthermore, they deduced from these experiments that the nerve fibres in the central end of the hypogastric nerve which subserve the reflexes must have their trophic centre, i.e., be connected with nerve cells either in the ganglion of the posterior root or in the spinal cord. Moreover when the first five lumbar nerves on both sides of the spinal cord were severed, centrally to the posterior root ganglion, and time allowed for degeneration to take place, reflexes

upon hypogastric nerve stimulation in a few, but not in the majority of cases, could be demonstrated. Hence these men were inclined to think that in the few cases where the reflexes persisted, they were due to some motor fibres running from the lower thoracic spinal nerves to the inferior mesenteric ganglion.

In the reflexes commonly seen upon stimulation of the hypogastric nerve, the impulses pass up the central ends of motor fibres which give off branches in the inferior mesenteric ganglion, to the nerve cells of the opposite hypogastric nerve and the reflex is an axon or pseudo-reflex and not a true reflex. In all the experiments cited, the axon reflex response was always a motor response. In 3 animals so studied Elliott (57) got contraction alone in 2 experiments and in one he observed contraction followed by relaxation. In the latter animal, stimulation of the intact right hypogastric nerve gave the same result. He therefore argued that there is no distinction between motor and inhibitor nerves in the hypogastric nerve trunk beyond that of the final area of distribution and that the ultimate response of the axon reflex may be either motor or inhibitory to the bladder.

Sacral nerve. Sensory fibres in pelvic nerve. Griffiths (211) found upon stimulation of one pelvic nerve (nervus erigens) a contraction of the bladder limited primarily to the side of the nerve stimulated. This was usually accompanied by expulsion of urine. Although the opposite half of the bladder contracted a little it invariably bulged outwards by the increased intravesical pressure caused by the forceful contraction of the other half. He was able to abolish the contraction of the half of the bladder opposite to that on which the nerve was stimulated in two ways; first by cutting the trunk of the opposite pelvic nerve and secondly by cutting the pelvic nerve itself proximally to the point of application of the stimulus. The contraction of the half of the bladder on the side opposite to that on which the nerve is stimulated is probably dependent upon a reflex through the lumbo-sacral part of the spinal cord, the efferent impulse passing along the opposite intact pelvic nerve to the bladder. This assumption was supported by the fact that section of either or both hypogastric nerves had no influence on its production.

The shortening of the bladder observed by Griffiths was attributed to a contraction of the external or longitudinally disposed muscular fasciculi and the indrawing was ascribed to the contraction of the inner obliquely and circularly disposed muscular fasciculi. These contractions therefore involved all layers of muscle in the wall of that side of the bladder.

The bilateral nature of the urinary bladder is well illustrated in the tortoise and in the common frog. In these animals the bladder is so deeply bilobed as to appear when distended like two sacs joined together at or near their necks. Each sac may contract independently of the other. We may assume, therefore, that the pelvic nerve contains both afferent and efferent fibres, a finding already reported by Budge (215). Stewart (232) reported similar conclusions, i.e., the hypogastric nerves do not carry motor impulses aroused by stimulation of any of the afferent fibres contained in the sacral nerves, but these impulses, if they cause reflex stimulation, do so over the efferent fibres contained in the opposite pelvic nerve. Denning (230), by calculation, believed that the pelvic nerve contains $\frac{1}{3}$ afferent and $\frac{2}{3}$ efferent nerve fibres. Macdonald and M'Crea (217) confirmed these findings. Stimulation of the intact pelvic nerve in the cat and dog caused strong contraction of the homolateral half of the bladder and a weaker contraction of the contralateral half of the bladder. But they observed occasionally in addition, relaxation of the bladder resembling a hypogastric rather than a normal pelvic effect. Cutting the hypogastric nerves had no effect upon the contralateral contraction of the bladder upon stimulation of one of the intact pelvic nerves.

A similar relaxation of the bladder following stimulation of the pelvic nerve was reported by Edmunds and Roth (209) in chloretonized cats. This was usually preceded by marked contraction, a change which can probably be explained on the basis of the anesthesia employed. From his observations upon the pelvic nerve, Griffiths (211) concluded that the hypogastric was not the sensory nerve from the bladder. Whatever the function of this nerve is, the normal action of the bladder may go on uninterruptedly, even when portions of both nerves have been excised and even though the nerve contains motor as well as sensory fibres. Of the two nerves he maintains the pelvic is the more important. He also studied the reflexes produced by stimulation of the three segments of the pelvic nerve to the bladder. In his experiments he showed that only that portion of the bladder is stimulated which is supplied by the particular segment excited. Thus if he stimulated the distal cut end of the middle segment only the middle portion of the bladder contracted. If, however, the middle branch is stimulated and its continuity maintained, the remaining branches being undisturbed, contraction of the entire half of the bladder is observed. If, however, the trunk of the pelvic nerve is cut and the central end of a divided branch is stimulated, contraction of the correspond-

ing lateral half of the bladder occurs. If both hypogastric nerves are cut in addition to the opposite pelvic nerve, but the pelvic nerve on the same side as that of the branch is intact, stimulation of the branch causes unilateral contraction of the bladder on the side stimulated. This is, according to Griffiths, a genuine reflex from the ganglia in the plexus and he is inclined to the view that ganglia on the pelvic plexus may act as reflex centres in addition to being trophic and distributing centres.

It is possible, however, that he is dealing with axon reflexes similar to those observed in the inferior mesenteric ganglion and the hypogastric nerves. As far as could be determined these findings have not been reinvestigated.

Hypogastric nerve influence. Sphincter urinae muscle, micturition and retention of urine. The literature on the sphincter urinae muscle, micturition and retention of urine is so involved and so much alike that I shall not attempt to discuss these separately in this review. In spite of the large number of researches which have been made on the urinary system there are still many differences of opinion in regard to some of the most important factors involved in the retention of urine and in micturition. All observers are agreed that both sets of autonomic nerves which supply the bladder have a motor effect on some part of the muscular wall of this organ, but that the pelvic nerves (nervi erigentes) exert the greater influence. Here, however, the agreement ceases.

The hypogastric nerves which supply the external genitalia originate in the male dog from the 12th thoracic to the 4th lumbar roots inclusive, in the cat from the 13th thoracic to the 5th lumbar roots and in the rabbit from the 2nd to the 5th lumbar nerve roots. A slight variation is found in the female. In the cat the 1st to the 5th lumbar nerves contribute fibres to its formation (229, 335). The cell relays for these fibres lie mainly in the inferior mesenteric ganglion (229).

Elliott (57) speaks of the cell relays of the inhibitory nerves for the bladder in the hypogastric nerves as lying chiefly in the inferior mesenteric ganglion side by side with those of the motor nerves of the urethra and base of the bladder. In anesthetized cats he passed a sound in the urethra toward the bladder. This tube extended the full length of the penile, membranous and prostatic urethra. A second sound was passed into the upper part of the urethra, through the bladder. One centimetre of urethra was left between the two sounds. The resistance to the passage of fluid was then studied in this segment. By raising or lowering the pressure within the upper tube it could be determined whether the resistance in this area was increased or decreased upon hypogastric and pelvic nerve stimulations.

It should be pointed out that the area selected by him is not commonly regarded as a part of the urethra and is not ordinarily differentiated from the vesical sphincter. Elliott reported the hypogastric nerves as causing constriction of the first part of the urethra which he says was far below the influence of changes in the bladder pressure. In the cat, it produced immediately constriction of the urethra of such power that the one centimetre of urethra could uphold a pressure of from 50 to 60 cm. water. The resistance yielded as soon as excitation of the nerve ceased. According to Elliott, it is only in the male that the urethra possesses a sheet of muscle with such powerful innervation. This he believes due to the fact that an unyielding constriction is needed to prevent regurgitation into the bladder from below the prostate when the secretion of the testicles is driven forcibly through the lower urethra against the resistance of the surrounding erectile tissue. In the female cat he observed the urethra capable of withstanding a pressure of only 35 cm. water upon hypogastric stimulation. Similar changes were noted in the dog, rabbit, ferret and other animals.

According to him, in the cat stimulation of the hypogastric nerve causes a rapid contraction of the urinary sphincter and relaxation of the fundus. Excitation of the pelvic nerve causes relaxation of the sphincter and contraction of the fundus. The two nerve trunks are completely antagonistic to one another. He, therefore, concludes that the pelvic nerves are the nerves of micturition and that the hypogastric nerves facilitate the retention of urine.

Fearnsides (283), in quoting Elliott, speaks of three types of action resulting when the sympathetics are excited: 1, constriction of the whole urethra and the whole surface of the bladder as seen in ferret and male goat; 2, constriction without shortening of the urethra with relaxation of the rest of the bladder in cat, pig, and monkey; and 3, contraction of the urethra and of a very small area at the back of the base of the bladder as seen in the dog, rabbit, female goat, and mongoose. In his studies he confirms Elliott but it should be pointed out again that the term sphincter was used as synonymous with the urethra (see table 3).

Although Gaskell (245) in 1887 was the first to show that stimulation of the pelvic nerve caused contraction of the detrusor urinae muscle and the hypogastric nerve contraction of the circular muscle fibres, it was v. Zeissl (291) in 1893 who, while endeavoring to establish the validity of von Basch's law in the bladder, investigated its innervation. He studied the innervation of the bladder in many dogs, and in these he varied the experimental procedures. He concluded that the pelvic nerve is

motor to the detrusor muscle and inhibitory to the sphincter. He also proved to his satisfaction that stimulation of the hypogastric nerve closes the sphincter and shuts off the bladder from the urethra. Both nerves contain motor and inhibitory nerve fibres for the bladder. The opening of the sphincter he believed is independent of the bladder con-

TABLE 3

This table gives the names of the authors and the results obtained by them on the detrusor and sphincter urinae muscles upon hypogastric and pelvic nerve stimulation.

− relaxation, + contraction, ++ strong contraction, 0 no effect.

NAME	HYPOGASTRIC NERVE		PELVIC NERVE	
	Detrusor	Sphincter	Detrusor	Sphincter
v. Zeissl	−	+	+	−
Beattle	−	+	+	−
Elliott	−	+	+	−
Frankl-Hockwort and Fröhlich	−	+	+	−
Langley and Anderson	+	++	+	+
Longet	+		+	+
Disse		+	+	−
Gaskell		+	+	
Fagge		+	+	0
Rehfisch	0	+	+	0
Wlasow		+		0
Courtade and Guyon		+	+	
Hanč*			+	−
Fellner	−	+	+	
François-Frank		+	+	
Denning	0	+	+	−
Stewart		+	+	
Griffiths	+		+	
Barrington	+		+	
		+	+	−

* Normal sphincter tonus maintained by central nervous system and any inhibition of the sphincter is central in origin.

traction and that it is actively relaxed by the pelvic nerve. His findings were later confirmed by Beattle (282), Courtade and Guyon (273), Elliott (57), and Frankl-Hochwart and Fröhlich (295).

In 1902 Fagge (199) repeated the experiments of v. Zeissl and obtained the same results. He, however, did not believe the experiments conclusive and he found no evidence to support the view that the pelvic

nerve inhibits the sphincter. The expulsion of urine caused by their stimulation always occurred at a pressure which was sufficient of itself to overcome the resistance at the neck of the bladder. Hypogastric stimulation caused contraction of the sphincter. When Fagge stimulated both sets of nerves he got no emission of urine even though the pressure in the bladder (22 cm. H_2O) exceeded that necessary to cause outflow under normal experimental conditions. When the pelvic nerves alone were stimulated, micturition occurred although the intravesical pressure reached a height of only 14 cm. of water. He concluded that the central nervous system is able to increase considerably the resistance to outflow of urine by means of impulses which pass down the hypogastric nerves. According to Elliott (57), Fagge's objections to v. Zeissl's theory may be explained by the fact that there is dilatation of the erectile tissue about the bladder neck.

Ott (285) observed the fact that 14 cm. water pressure was necessary in the bladder of the cat to cause fluid to leave the urethra. Upon cutting the lumbar nerves, the intra-vesical pressure necessary to cause a flow of fluid from the urethra decreased to 2 cm. water pressure. In the same animal after death the pressure necessary was found to be 1 cm. Somewhat similar findings were recorded by Heidenhain and Colberg (299). They found the intra-vesical pressure necesssary to cause urine to leave the bladder by way of the urethra in the living animal to be 9.4 cm. and in the dead animal 5.8 cm. water. Barrington (241) used female cats for experimentation. In these the entire fundus of the bladder was cut off and the remaining muscle coats stripped off the beginning of the urethra. A tube connected with a pressure bottle was tied in the remaining portion of the bladder which then consisted of a mucous membrane sac. He noted the pressure at which fluid would flow through the urethra. In all instances stimulation of the hypogastric nerve caused contraction of the urethra followed by relaxation and increased flow of fluid from the urethra.

These contentions were immediately doubted by Langley and Anderson (229). The lumbar nerve, they found, caused contraction of the whole musculature of the bladder. The contraction is strongest at the base of the bladder near the entrance of the ureters (trigone). Stimulation of the nerves on one side causes contraction of both sides. They suggest that the stoppage of flow obtained by v. Zeissl on stimulation of the hypogastric nerve might possibly be due to a longitudinal contraction of the basal portion of the bladder drawing the urethra forward. They hold that the theory, that the lumbar nerves cause contraction of

the circular coat and not that of the longitudinal coat, is untenable since the longitudinal fibres are obviously continuous with the bundles of the oblique and of the circular muscle fibres. Similar views were expressed by Longet (296) and Griffiths (211). The failure of these observers to note contraction of the inner circular muscle of the urethral segment, Elliott believes, was due to the fact that they employed direct inspection and not mechanical devices for detecting the changes produced.

Griffiths (211) could find no definite accumulation of circular muscle fibres at the neck of the bladder which might be regarded as a special sphincter. He found the resistance to the outflow of urine in the urethra to be the same after division of both hypogastric nerves and even after death. He maintains that under ordinary circumstances the unstriped muscular wall of the urethra does not relax actively while the bladder contracts to expel its contents but simply yields to the pressure and that contraction of the muscle takes place at the end of micturition. A special muscular mechanism would thus not be necessary in normal retention of urine. Other observers had already voiced similar views. Mosso and Pellacani (281) in 1882 concluded that the resistance of the "sphincter" is tonic and constant and that emission of urine takes place only when the sphincter yields to a pressure sufficiently great to overcome its tonic contraction.

Budge (215) maintains, since a true vesical sphincter has no share in normal retention of urine in the bladder, that the term sphincter is a misnomer. Clark (294) pointed out that the smooth muscle of the bladder is under the control of the will as well as under reflex influence. In early life, he said, it is chiefly under reflex influence but it is gradually rendered voluntary by education and habit. He did not believe the annular fibres around the neck of the bladder are a sphincter. If his theory is correct, that this smooth muscle can be influenced voluntarily, it is as far as we know the only sphincter in the body consisting of smooth muscle and innervated by the autonomic nervous system, which is voluntarily controlled.

Hanč ascribes the reflex inhibition of the sphincter to the intervention or inhibition by efferent nerve impulses. He found the latent period of the sphincter to be longer than that of the bladder and not dependent upon the bladder pressure. Goltz (288) also ascribed the emptying of the bladder to a reflex centre in the lumbar area. And Gianuzzi (239) placed the centre for tonus of the vesical sphincter at about the level of the 5th lumbar root. The normal sphincter tonus, Rehfisch (234) believed, is maintained by the central nervous system. The inhibition of the sphincter he ascribes to an inhibition of this central tonus.

Sensory nerves in the bladder are stimulated by the distention of the bladder and these impulses stimulate the motor nerves in the lumbar sacral region in the cord, causing micturition. He investigated this phenomenon in 45 male dogs. He was able to show the action of the hypogastric nerve upon the "sphincter." If, when the intra-vesical pressure was high though not high enough to cause urine to flow from the urethra, the hypogastric nerves were cut urine immediately began to flow through the urethra. If the distal cut, end of one hypogastric nerve was then stimulated, the flow of urine immediately ceased, proving, he believed, the importance of the hypogastric nerve in the normal retention of urine.

Guyon (298) noted that the reflex of micturition was unaffected by sectioning of the hypogastric nerve but that it disappeared when the pelvic nerves were cut.

Beattle (282) finds the involuntary muscle of the urethra innervated both from the hypogastric and the pelvic nerves, but mainly from the latter. Section of the hypogastric nerves in dogs, cats and human beings produces no interference with normal micturition. Section of the sacral or pelvic nerves abolishes all sensation in the bladder in dogs and in cats and in these, retention with over-flow occurs. After some days the over-flow ceases and the animal passes urine in small quantities voluntarily, but a large amount remains in the bladder. If the pudic nerve is now cut the voluntary micturition is abolished and continual dribbling results. Upon section of the pudic nerve alone micturition remains practically normal. He discusses in considerable detail various centres for micturition but such a discussion has no place in this report.

The arguments, upon which the theory of sphincter action of the circular muscle in the bladder is founded, are weak. If one considers the circular coat as a continuation of the longitudinal muscle layer, no true sphincter exists. There seems little doubt, however, of the existence of a centre in the lumbar cord which regulates the urine flow and there is no question as to the action of the pelvic nerve upon the longitudinal muscle of the bladder. The bulk of the evidence supports the view that the hypogastric nerves increase the resistance to the passage of urine at the urethral orifice in some way. The question therefore arises as to how the orifice dilates in micturition. There are three possible ways in which this may occur. It may be dilated: 1, passively by the pull of the contracting bladder and increased intra-vesical pressure; 2, by inhibition of the impulses over the hypogastric nerves through the lumbar centre, i.e., inhibition of the tonic contraction in the neck of the bladder; 3, by

active inhibition of the muscle itself, through impulses sent over the pelvic nerves. There are experiments in the literature supporting each of the above views. It is possible that all of these are combined in micturition.

Vasomotor changes. Much to my surprise in going over the literature I find that no actual experiments and only a few incidental observations have been made on the vasomotor supply to the bladder in mammals. Young (163) claimed that both systems, hypogastric and pelvic nerves, carry vasomotor fibres to this organ. Langley and Orbeli (252) reported vasomotor fibres as well as vesico-motor fibres in the 6th, 7th, 9th, and 10th nerves in the frog. Waters (253) found an indifferent action of the 8th nerve in the frog which at times caused contraction of the blood vessels of the bladder. Langley and Anderson (229, vol. xix), found the lumbar set of nerves to have very little, if any, effect on the arteries of the bladder. Occasionally the hypogastric nerve seemed to produce slight vaso-constriction of the smallest arteries and slight pallor, but in most cases there was undoubtedly no effect since they usually saw the small arteries pulsating in undiminished extent. This was the more striking since the small arteries of the intestine, of the uterus and of the penis disappeared from view completely in a few seconds upon stimulation of the hypogastric nerves.

Since both the hypogastric and pelvic nerves contain nerve fibres with diameters of 1.3 to 4μ and since Gaskell (245) has shown that the vasomotor fibres are the finest medullated fibres in the splanchnic nerve (1.8 to 3.6μ) we may assume from analogy that both autonomic nerve trunks carry vasomotor fibres to the bladder wall.

Pharmacology. As said before (p. 507) epinephrine and ergotoxin are regarded as typically sympatho-mimetic drugs and pilocarpine and atropine are usually regarded as parasympatho-mimetic in action. The effects of these drugs on the urinary bladder have been extensively studied. From table 4 it will be seen that the response of the bladder to epinephrine is identically the same as to stimulation of the sympathetic nerve trunk, or the hypogastric nerve. The responses vary in different individuals of the same species and in different species in the same way as do the responses to stimulation of the nerve. This variation was first studied by Elliott (57) on a large group of animals, although Dale was the first to note the action of epinephrine on the bladder.

We have already, in our earlier reference to ergotoxin, alluded to its specificity for sympathetic motor nerve endings. By its use it is possible to demonstrate the presence, not only of motor nerve fibres in the hypo-

TABLE 4

A table giving the names of the investigators and the experimental animals each used in his work

The effects of hypogastric nerve stimulation and of exposure to epinephrine upon the urinary bladder before and after the use of ergotoxin are here presented.

C contraction, R relaxation, C + R contraction followd by relaxation.

AUTHOR	YEAR	ANIMAL	HYPOGASTRIC	EPINEPHRINE	EPINEPHRINE AFTER ERGOTOXIN	STIMULATION OF HYPOGASTRIC NERVE AFTER ERGOTOXIN
Dale	1906	Cat	C + R	C + R	R	C
		Cat	C + R	C + R		
		Monkey	C + R	R		
		Rabbit	C	C		
		Guinea pig	Indifferent	Indifferent		
		Hedgehog	Indifferent	Indifferent		
		Rat	Indifferent	Indifferent		
Elliott	1905	Dog	C	C	R	C + R†
		Ferret	C	C	R	R
		Indian Civet cat	C	C		
		Pig	C + R	R		
		Male goat	C	C		
		Female goat	C	C		
Barger and Dale	1907	Pig		R	R	R
		Goat		C	R	R
Chiari and Fröhlich	1911	Cat	R			
Adler	1918	Frog	R			
Abelin	1919	Rabbit	R	R‡ C + R§		
Edmunds and Roth	1920	Cat	C + R	C + R		
Macdonald and M'Crea		Cat	C*	C*	R	R
			R	R		
		Dog	C*	C*	R	R
			R	R		
Dale	1906	Ferret	C	C	R	C
Streuli	1915	Rabbit		C or R	R	

* In animals under light anesthesia.

† Relaxation in bladder previously treated with pilocarpine.

‡ Excised bladder.

§ Intact bladder.

TABLE 4—*Concluded*

AUTHOR	YEAR	ANIMAL	HYPOGASTRIC	EPINEPHRINE	EPINEPHRINE AFTER ERGOTOXIN	STIMULATION OF HYPOGASTRIC NERVE AFTER ERGOTOXIN
Elliott	1905	Mongoose	C	Indifferent		
Dale	1906	Dog	C	C	R	R
Dixon	1903	Cat	C + R	R		
Langley	1901	Cat		R		
Rose and Deakin	1928	Dog		C with small doses	R	
				R with large doses	R	
Macht				R		

gastric nerve to the bladder, but also of inhibitory fibres. In the early reports Dale (242) called this substance chrysotoxin but he later renamed it ergotoxin when he found them to be the same substances (300).

Following the administration of ergotoxin the response of the bladder to epinephrine may remain the same or may be reversed. If the response of the bladder to epinephrine normally is inhibition, ergotoxin effects no change in the epinephrine reaction. If the bladder normally responds to epinephrine by contraction or contraction followed by relaxation (see table 4) ergotoxin paralyzes the motor fibres and epinephrine causes only relaxation of the organ since only the inhibitory sympathetic fibres are sensitive to the epinephrine. A similar reversal of action of the organ following the administration of ergotoxin is noted when the hypogastric nerves are stimulated. The authors are not entirely agreed on this point. Dale (242) noted no change except a decrease in the extent of contraction upon stimulation of the hypogastric nerve, following the administration of ergotoxin although epinephrine caused relaxation following ergotoxin.

According to Macdonald and M'Crea (217), epinephrine affects the bladders of the dog and cat just as does sympathetic stimulation, i.e., it causes a slight relaxation followed by an increase in tone and contraction. The contraction is more prolonged than that produced by sympathetic stimulation. They noted the fact that 0.002 mgm. of epinephrine caused a fall in blood pressure and a marked contraction of the bladder in animals under light ether or chloralose anesthesia. If the anesthetic was deep, relaxation of the organ was noted. They obtained, not only

an increase in general tone in these cases, but also an increase in the rate and force of the contractions. These changes were not noted when ephedrine was similarly administered. They also recorded a reversal in action of epinephrine following the injection of ergotoxin. If in cats and dogs under light ether anesthesia or chloralose anesthesia, or in decerebrated or spinal animals in which epinephrine had caused increased tonus, a second injection of epinephrine followed the administration of ergotoxin, a reversal of action, i.e., a relaxation of the bladder muscle always occurred. They gave an entirely different explanation of this reversal of action than did Dale and Elliott.

It is agreed that epinephrine acts upon the neuro-muscular mechanism, or synaptic substance of Langley, which according to the best authorities is neither muscle nor nerve. In a denervated cat bladder (denervated by cutting the hypogastric nerves and allowing time for degeneration to take place) epinephrine proved still an effective stimulus, although it less readily excited the organ and the response when it occurred was more prolonged than under normal conditions.

Edmunds and Roth (209), Young and Macht (305), and Macht (110) cut strips from various parts of the bladder to determine whether adrenalin acts on all parts. Elliott (57) had previously stated that the sympathetic nerves are distributed to the bladder mainly in the wall between the openings of ureters and extend for varying distances over the dorsal surface and that the pelvic nerves supplied other parts of the bladder. Both Edmunds and Roth and Young and Macht reported that strips taken from the trigone were more sensitive to epinephrine than other parts of the bladder. In Edmunds and Roth's experiments strips taken from the trigone of the cat's bladder contracted when epinephrine was added to the bath, whereas segments taken from other parts of the bladder relaxed. They state, however, that the contraction of the trigone occurred only with extremely dilute solutions and that stronger solutions caused relaxation. They explained Elliott's failure to obtain contraction in the trigone to his having used too great concentrations of the drug.

Using isolated rabbits' bladders which had been treated with pilocarpine in order to give them tone Streuli (301) found that epinephrine gave complete relaxation and stopped the automatic movements even in dilutions as great as 1:40,000,000. Epinephrine caused contraction of the organ after the administration of atropine. He also noted that subminimal doses had a tendency to increase the tone of the bladder especially if the original tone was low. He also made the interesting ob-

servation that a bladder placed in Tyrode's solution in which the calcium content was above normal relaxed more readily upon the addition of epinephrine to the solution than in Tyrode's with normal calcium content. Ergotoxin had no influence upon the inhibition of the bladder by epinephrine but it did diminish the response to pilocarpine. Chiari and Fröhlich (302) made similar observations, i.e., they reported the fact that removal of calcium from the blood in the cat or decreased calcium in the bath surrounding the isolated organ increased the sensitivity of the organs, urinary bladder, etc., to impulses of the vegetative nervous system and the effect of stimulation of these nerves can be decreased by an excess of calcium. Thus, after excess of calcium, relaxation of the bladder always occurred when epinephrine was either injected intravenously or added to the bath in which the muscle was contracting.

Pilocarpine-atropine. The results reported on the action of pilocarpine and atropine on the bladder are uniformly the same. In all cases pilocarpine causes an increase in tone and contraction of the organ (209, 217, 229, 297, 301–307), similar to that commonly produced by stimulation of the pelvic nerve. Because of this similarity and because in other organs innervated by the parasympathetic system, pilocarpine stimulates the neuro-muscular junction or "nerve muscle complex" of this system many men regarded its action as being upon the same mechanism in the bladder. This inference is supported by the fact that atropine antagonizes the action of pilocarpine. However, some doubt has been cast upon the point of action of these drugs since atropine completely antagonizes pilocarpine, but does not in these animals prevent the contraction produced by stimulation of the pelvic nerve (209, 303–305). If atropine paralyzes the parasympathetic neuro-muscular junction, pelvic nerve stimulation as well as pilocarpine should be ineffective. Langley and Anderson (229) did record a decrease in contraction upon excitation of the pelvic nerve following an injection of atropine. They assumed that pelvic nerve excitation was a superior stimulus to pilocarpine excitation.

From these findings we may infer from pharmacological as well as physiological experiments that the bladder is innervated by the sympathetic and parasympathetic systems, each supplying both motor and inhibitor impulses and that there is no difference between the effects of these two systems, except the possibility that the parasympathetic nerves carry stronger motor impulses.

EXTERNAL GENERATIVE ORGANS. The outstanding investigations in this field are still those published by Sherrington (243) and Langley and

Anderson (229, vol. xix). The parts of the generative organs to be considered here are those which are developed from the uro-genital sinus and the skin surrounding the uro-genital opening. In the female they are the vulva, the vestibule, extending a short distance above the urethral opening (in the human also the vagina) and the genital skin. According to Langley and Anderson, the vagina does not belong to the external generative organs, developmentally or by its innervation. In the male these parts are the penis, the scrotum and in some cases other parts of the skin in the genital region.

URETHRA. The urethra varies in length in the sexes and also in individuals of the same sex. In the male it is commonly divided into the prostatic, membranous and penile or spongy portions. Bundles of nerve fibres are found between the longitudinal and circular muscle fibres and also in the submucosa (16). Multipolar ganglion cells are found in the adventitia. In man most ganglion cells are found in the neighborhood of the prostatic urethra, but in the female they are most abundant in the adventitia.

Sensory end organs are found in the skin and in the male in the glans penis and in the female in the clitoris. The urethra receives autonomic fibres from three sources: the hypogastric, the pelvic and the pudic nerves.

THE HYPOGASTRIC NERVES. If one considers the effects on the urethra already cited (p. 539), as acting primarily on the "sphincter" then only a few experimenters have noted changes other than vasomotor phenomena in the urethra upon hypogastric nerve stimulation. Upon anatomical dissection, Loven (292) in 1866 showed the hypogastric nerves to innervate the urethra. Langley and Anderson (229, vol. xix) observed slight movement of the penis and contraction of the vas deferens upon excitation of this nerve. But their attention had been focussed again mainly on the vasomotor effects of hypogastric nerve stimulation. In some cases they found that stimulation caused slight shrinking of the penis. If a pelvic nerve was stimulated to obtain marked erection of the penis, subsequent stimulation of the sympathetic nerve caused some shrinking and on further stimulation of the hypogastric nerves marked shrinkage of the penis was noted. In a later paper they find the general effects of stimulation of the hypogastric nerve the same in the rabbit, the cat and the dog and in all cases unilateral stimulation had a bilateral effect, although the effect was greater on the stimulated side. The outstanding change was that of contraction of the arteries both large and small especially in dogs. The penis decreased in size and pallor occurred.

There was also contraction of the arteries of the whole genital skin. This was especially noticeable in the scrotum of the rabbit. Contraction of the intrinsic smooth muscles of the penis itself apart from those caused by the retractor penis occurred. The most obvious change was the shrinking of the bulbus urethra. Contractions of the unstriped muscle of the genital skin also occurred and contraction of the scrotum and cremaster muscle.[2]

In the female, they report upon hypogastric nerve stimulation changes "mutatis, mutandis the same as in the male." They observed pallor of the clitoris and of the mucous membrane of the vulva with slight retraction of the clitoris, contraction of the vulva, and contraction of the skin muscles so as to draw the vulva dorsally.

François-Frank (293) studied the action of the hypogastric nerve upon the volume, the arterial blood pressure, venous pressure and blood flow in the penis of the dog. From his results he concluded that through it both vaso-constrictor and vaso-dilator nerve fibres are supplied to this organ although the predominant action is that of vaso-constriction.

Contraction of the prostatic, membranous and bulbous portions of the urethra was observed by Fagge (199) upon stimulation of the hypogastric nerve. And Young (163) noted contraction of the involuntary muscle of the urethra upon hypogastric stimulation.

The pudic nerve, an ordinary skeletal nerve, originates from the 1st, 2nd, 3rd, and 4th sacral roots (211, 282). In the dog it corresponds practically to the internal pudic nerve in man, but not in the rabbit (229, vol. xix). The fibres in its trunk are almost all large medullated fibres measuring 12μ in diameter but there are also many small medullated fibres measuring 2 to 3μ. The smaller fibres are probably the vasomotor and the motor and inhibitor autonomic nerve fibres to the smooth muscles. In addition to the efferent fibres it contains afferent (sensory) fibres to the prostate gland, the genital portion of the urethra, the end of the penis in the male and clitoris and vulva in the female. These afferent fibres can be readily demonstrated in the experimental animal. If one sections the pudic nerves and stimulates the central end of one trunk contraction of the urinary bladder occurs. If the bladder is already in a contracted state slight contraction followed by relaxation is produced (211).

Langley and Anderson (229, vol. xix) studied the action of the pudic nerve in cats, dogs and rabbits. In these they observed the fact that

[2] Of the experimental animals used, only the dog has a cremaster muscle, Langley and Anderson.

stimulation of the cut peripheral end produced contraction of the tunica dartos of the scrotum, and quick projection of the penis, which soon became blue, then pale and then slowly retracted.

More definite information concerning the action of the pudic nerves is presented by Young (163), Gaskell (245), and François-Frank (293). According to Young and François-Frank, vaso-constriction in the erectile tissue, the penis becoming small and flaccid, is one of the outstanding actions of these nerves. In addition the striated urethral muscles contract (283, 163). When these nerves are sectioned erection does not occur, sensation is lost in the organ so that mechanical stimulation does not cause either erection or ejaculation and there is a tendency to incontinence and dribbling even if the other nerves remain intact.

Barrington (241) also noted a decreased flow of urine through the urethra upon stimulation of the pudic nerves. This was not due to contraction of the striated muscle in his experiments, but to contraction of the smooth muscle between the bladder proper and the prostate portion of the urethra.

Effect of stimulation of spinal cord. That responses to the autonomic nerves can be influenced by stimulation of the spinal cord, section of the spinal cord and excitation of spinal mixed nerve has been known almost 100 years. In 1837 Brachet (308) observed ejaculation in rabbits upon stimulation of the spinal cord. Two years later he demonstrated erection of the penis upon section of the spinal cord in the lumbar region and ejaculation occurred following rubbing of the organ with the prepuce. His results were supported by those of Hall (309) experimenting upon a man in whom an accident had caused a broken back and by those of Goltz and Freusberg (288) who showed the existence of a similar erector centre in the lumbo-sacral region of the spinal cord in the dog. Goltz sectioned the cord in dogs under chloroform anesthesia between the last thoracic and the 1st lumbar vertebrae. After the animal recovered from the anesthetic, rubbing the foreskin very gently back and forth caused erection of the penis. Stimulation of the bladder caused a similar erection. After extreme erection was established stimulation of the foot, the sciatic or any cutaneous nerve caused the organ to become flaccid at once. Elliott (57) noted a similar reflex action, i.e., if the central end of the cut great sciatic nerve was stimulated, relaxation of the urethra was noted, but if the proximal end of the cut small sciatic or pudic nerve was stimulated, contraction occurred. He advanced no theory as to the cause of this difference. Goltz also noted that destruction of the cord below the cut caused permanent disappearance of the reflex.

Spina (310) has shown that if the spinal cord of a male guinea pig is transected between the 12th dorsal and 1st lumbar vertebrae spontaneous erection occurs, followed by expulsion of semen. Akutsu (311) observed in guinea pigs ejaculation without erection upon stimulation of pre- and post-ganglionic fibres of the hypogastric nerve. Working upon these findings Bacq (312) was able to render male rodents, guinea pigs, rabbits, and rats, impotent sexually upon sympathetic denervation of the genital organs. In the early acute experiments, after sympathectomy, the spinal cord was transected at the level of the last costo-vertebral articulation and although erection occurred, ejaculation was never observed. From these experiments, it is seen that removal of the sympathetic innervation to the genital organs of the male guinea pig prevents ejaculation but not erection. He later sympathectomized male rats, guinea pigs, and rabbits. When these were placed with female animals copulation occurred. The orgasm of falling on his side and uttering a cry during copulation noted in the normal male rabbit was never seen. Bacq was never able to find semen in the vagina of the female animal. Thirty-seven days to two months after sympathectomy, the males again became potent. This he believed due to regeneration of the sympathetic nerve fibres.

Pelvic nerve. The lower set of nerve fibres which innervate the external genitalia are derived from the 1st, 2nd and 3rd sacral nerves in cats and the 1st, 2nd, 3rd, 4th, and 5th sacral nerves in the rabbit (229). According to Elliott (57), stimulation of these nerves causes relaxation of the urethra, a relaxation involving not only the circular muscle coat, but also the longitudinal coat. Barrington (241) confirmed these findings.

The origin of the inhibitory fibres in the external generative organs is the same in the male and female. According to Langley and Anderson these inhibitory fibres have their origin in the dog and cat in the 1st, 2nd, and 3rd sacral roots and in the rabbit from the 2nd, 3rd, and 4th anterior sacral roots. Sherrington (243) noted imperfect turgidity of the penis in the monkey upon stimulation of the anterior roots of the 8th and 9th subthoracic nerves. This imperfect erection he believed was due to the deep anesthesia and the very young, sexually immature animals used. In the cat he observed turgidity upon stimulation of the 8th and 9th anterior roots and to a less degree the 7th root. These results do not agree absolutely with those of previous observers. Eckhard (246) was the first to show that certain nerves when stimulated caused enlargement of, and increase of blood flow through, the penis. These nerves he called

the nervi erigentes. Langley and Anderson (229) who later confirmed his findings called them the pelvic nerves. This is the form now most commonly used. The origin of these nerves Eckhard found to be from the 1st and 2nd anterior sacral roots and occasionally from the 3rd in the dog. Nikolsky (237), shortly thereafter, showed the 1st branch to contain both vaso-constrictor and vaso-dilator fibres and that the 2nd branch was purely a vaso-dilator nerve trunk. Gaskell (245) noted the erector fibres for the penis to leave the spinal cord by way of the 2nd and 3rd sacral nerves in the rabbit. Morat (313) confirmed Eckhard's findings in the dog. He observed upon stimulating either the 1st or 2nd sacral roots a change in the size, consistence and color of the organ. François-Frank studied the vaso-motor innervation of the penis in dogs using as his criteria changes in the volume, arterial pressure, venous pressure and rate of blood flow in the organ. He arrived at the conclusion that the 1st sacral root carries both vaso-constrictor and vaso-dilator fibres, whereas the 2nd branch contains vaso-dilator only. The vaso-constrictor fibres, however, he maintained are not typically those of the sacral nerve but are an accretion of fibres from the sympathetic system. Fröhlich and Loewi (307) also concluded that the pelvic nerve contained both vaso-dilator and vaso-constrictor nerve fibres, but the vaso-constrictor action was overbalanced by the vaso-dilator effect.

Inasmuch as the pelvic nerves are capable of causing contraction of the bladder, relaxation of the sphincter and erection of the penis in the male, Young (163) points out the fact that because no erection occurs during micturition, only a part of the fibres in the nerve trunk can be active. If erection had occurred emptying of the bladder quickly subdued it.

Pharmacology. Within limits the above findings upon autonomic nerve stimulation have been confirmed by the use of sympatho-mimetic and para-sympatho-mimetic drugs.

Elliott (57) noted that in the rabbit, an administration of epinephrine caused a contraction of the urethra sufficient to hold up a pressure of $\frac{1}{2}$ meter of water. In the cat and dog the pressure sustained after an administration of epinephrine amounted to 50 to 60 cm. of water.

In the dog marked paling and a decrease in the diameter of the penis is noted following the intravenous injection of epinephrine. The time spent between the injection and the vasomotor response is usually longer than that noted in other visible cutaneous vessels and the duration of action is also longer. These results support the conclusions as to the presence of vaso-constrictor fibers in the penis.

In 1879 Nikolsky attempted to show that pilocarpine, by acting upon the pelvic nerve endings, caused vaso-dilatation of the dog's penis. The increased blood flow could be decreased by the administration of atropine. He also showed that stimulation of the pelvic nerve after atropine had no influence upon the organ. Ostroumoff (314), Anrep and Czybulski (315), Piotroski (316), Langley and Anderson (229, vol. xix), and others failed to confirm these findings.

RETRACTOR PENIS MUSCLE. The retractor penis muscle which is an active structure in the male rat, dog, cat, horse, hedgehog, and other animals has been used in studying the autonomic innervation of the external generative system (246, 229 and others). In the female a corresponding muscle is the caudo-vaginal muscle (317). In early reports the muscle was considered to be a thin band of longitudinally arranged unstriated muscle, but this mistake was corrected by Fisher (318). In a medium size dog it is about 5 cm. long with a diameter of about 3 mm. (318–320). The anterior $\frac{3}{5}$ of the muscle in the dog at least is composed of non-striated muscle, but the posterior $\frac{2}{5}$ consists of equal amounts of non-striated and striated muscle fibres. Fisher was unable to find nerve ganglia in either the muscle or in its sheath. Fletcher describes a superficial plexus formation on the surface of the muscle in which the fibres branch freely, but do not anastomose. Placed in close approximation to the fibres and most commonly at the nodal points of the plexus are numerous oval nuclei (10 to 13μ by 3 to 5μ) which (317) resemble those in the sheaths of the main nerve fibres. From the plexus formation fine branches pass inwards to form an intercellular network and according to Fletcher this network is very elaborate and close set, the muscle cells being embraced on all side by a labyrinth of the finest branches of nerve fibres. There is no evidence that special endings are present nor that nerve endings enter the muscle cell substance.

The retractor penis muscle receives its autonomic innervation from nerve fibres in the lumbar and sacral nerve roots. According to Langley (229, vol. xix) the hypogastric originates in the dog and cat from the 2nd, 3rd, and 4th lumbar nerve roots with some minor variations (see p. 522). In the hedgehog, Fletcher (317) observed the motor supply to be contributed by the 12th and 13th thoracic and upper two lumbars. These have a long post-ganglionic course by way of the gray rami to the upper lumbar nerves and the pudic nerves. From the pudic nerve the motor fibres reach the retractor both by the genito-anal branch and by the nervus dorsalis penis. The inhibitory autonomic fibres are derived from the upper sacral roots and are contained in the pelvic nerve. These

have a short post-ganglionic course from the first pelvic ganglion to the retractor muscle.

It is agreed that stimulation of the upper roots of the sympathetic or the pudic nerve causes the vessels to become nearly empty and the retractor penis muscle contracts powerfully (229, 317, 319, 205, 321, 320, and others). Stimulation of the pelvic nerve, on the other hand, causes dilatation of its vessels and relaxation of the muscle (229, 307, 317, and others).

The results obtained upon the intravenous injection of epinephrine or its addition to the bath in which the segment of muscle is immersed confirm the experiments on nerve stimulation. In all instances epinephrine causes powerful contraction of the retractor penis muscle which is more powerful than that obtained upon stimulation of either the pudic or sympathetic nerves. If the effect of epinephrine is tested again after the administration of ergotoxin, contraction is not observed but relaxation usually results (320). Elliott (57) has shown that the denervated retractor penis muscle in the dog and rat is readily excited by epinephrine to stronger contraction. Here again are demonstrated both inhibitory and motor fibres in a single autonomic nerve trunk.

The results obtained with those drugs commonly called parasympathomimetics do not conform to those obtained upon stimulation of the pelvic nerve. Indeed, instead of getting relaxation of the muscle, by the administration of pilocarpine, muscarine or physostigmine, contraction is obtained (319, 278, 307, 320, and others). Atropine, true to form, acts antagonistically to pilocarpine. Only Botazzi (278) observed it to excite the muscle. Although it counteracted the effect of pilocarpine, it had no effect upon the results of pelvic nerve stimulation.

Edmunds (320) used the anterior $\frac{2}{3}$ of the muscle from the dog. This strip was immersed in a bath of Ringer's solution and spontaneous rhythmic contractions, if not always present, were initiated by the addition of pilocarpine to the bath; if present they were strengthened. He never saw elongation of muscle as described by Botazzi (278).

The point of action of these drugs is discussed in detail by Edmunds. Suffice it to say that pilocarpine still remained active when administered after ergotoxin and it therefore probably does not act upon the sympathetic neuro-muscular junction, but upon the muscle substance proper. In the light of Macdonald and M'Crea's work (217), the question arises as to whether the retractor penis muscle is innervated by both inhibitor and motor parasympathetic fibres. If so, do the more powerful motor parasympathetic fibres in the pelvic nerve predominate over the weaker inhibitor fibres upon pilocarpine administration?

PROSTATE GLAND. Structurally, the prostate gland consists of glandular epithelium, connective and non-striated muscular tissue. The smooth muscle is disposed chiefly around the acini of the gland, about which it is arranged both longitudinally and circularly (323, 328). Imbedded in the substance of the organ and in its capsule are numerous nerve cells and ganglia (16). The number of lobes in the gland varies with the species of animal.

The function of the prostate gland is more complex in some animals than in others (324). Its musculature contracts during ejaculation, forcing its own secretion into the urethra, and aiding in the propelling of the seminal fluid along the urogenital tract.

According to Fearnsides (283) the afferent channels to the prostate and prostatic urethra are related to the 10th, 11th, 12th thoracic roots and also the 1st, 2nd, and 3rd sacral nerve trunks. The efferent fibres are derived from the same nerve trunks as those of the bladder (see table 2).

In 1863 Eckhard (246) determined what the autonomic innervation of the prostate gland was. He was able to produce ejaculation of prostatic secretion on stimulating the pelvic (nervus erigens) nerve. Continuous stimulation, however, failed to produce further secretion. His observations indicated that the pelvic nerves supplied motor fibres to the smooth musculature in the gland but not secretory fibres to the glandular portion of the gland. The hypogastric nerves carry both kinds of fibres, secretory and motor.

His results were partly confirmed by Langley (228) who observed the prostate to behave like the uterus to stimulation of the sacral and lumbar sympathetic nerves. In a later article, Langley and Anderson (229, vol. xix) reported contraction of the muscular sheath of the prostate gland in the dog upon stimulating the hypogastric nerve. Similar reports were later presented by a number of investigators (163, 211, 325).

Mislawsky and Bormann (326) obtained only temporary secretion upon stimulation of the pelvic nerve. Stimulation of the hypogastric nerves caused a continuous secretion which they likened to the salivary secretion upon stimulation of the chorda tympani nerve. After cessation of hypogastric stimulation, excitation of the pelvic nerve again caused secretion. They concluded that the pelvic nerves merely press out the secretion formed in the gland tubules by contracting the smooth muscle found in the gland itself. These investigators noted pilocarpine to excite the gland to secretion and this could be further augmented by hypogastric nerve stimulation. Atropine caused complete suppression

of the secretion induced by pilocarpine and subsequent stimulation of the hypogastric nerve had no effect. They maintained that atropine paralyzes the sympathetic nerve endings and that the prostate gland is devoid of parasympathetic terminals.

Waddell (323) excised the prostate gland in a number of animals (rats, guinea pigs, cats, hogs, rabbits) and studied the effect of drugs upon these excised organs. Epinephrine caused contraction in all cases (114). Pilocarpine caused a shortening of the strip which could be antagonized by atropine in the rabbit's prostate only. He concluded that the prostate possesses a motor parasympathetic as well as a motor sympathetic innervation. This work was later extended by Macht (327) using these same and other animals. Like Waddell, Macht observed contraction of the prostate gland when epinephrine was added to the bath in which the organ was immersed. If ergotoxin was first added and epinephrine added later, the action was purely that of relaxation. Except in the rabbit pilocarpine had no effect. He concluded that the prostate possessed a true sympathetic innervation, but, of the animals studied, only the rabbit, if any, possessed a parasympathetic innervation of the prostate.

Cowper's glands. The innervation of Cowper's glands is also by way of the hypogastric nerves (325), and according to Timofejew (330), they are both vaso-motor and secretory in function.

Cremaster muscle and scrotum. Very little information on this subject is available in the literature. Sherrington (243) has shown that excitation of the 2nd and 3rd lumbar anterior roots in the Rhesus monkey throws the cremaster muscle into contraction. When the testicle is descended, it is raised on excitation of these roots. Langley and Anderson (229) have noted contraction of the cremaster muscle in the dog and contraction of the scrotum in the cat, dog and rabbit. They saw the cremaster muscle contract upon stimulation of genito-crural nerve. They also noted that epinephrine contracts these structures.

Internal generative organs. In referring to the internal generative organs, we mean those organs which are not developed from the uro-genital sinus or from the surrounding skin, but from either the Müllerian or Wolffian ducts. In this group are included the ovaries, testes, uterus, vagina, fallopian tubes, vas deferens, and seminal vesicles (uterus masculinus).

Testes. Stöhr (16) believes the autonomic nerves for the testes originate in the 10th thoracic segment. These fibres pass through the coeliac, aortic and renal plexuses ultimately forming the spermatic

plexus and fibres from this plexus and that of the plexus deferens supply the testes. No ganglia have been shown to be present in the organ. Of what happens to these nerve fibres after reaching the organ apparently little is known. Retzius (329) and Timofejew (330) traced some of these fibres to the blood vessels around which they form a dense plexus. They also believed they traced a few fibres to the tubules, though the latter finding was not supported by Stöhr (16). Since these plexuses are formed about the blood vessels, it is natural to assume that these nerves are concerned with the vaso-motor responses observed in the organ. This inference is supported by the findings of Langley and Orbeli (252), working upon the frog. They noted that sympathetic stimulation caused contraction of the blood vessels of the testes in this animal and that the spinal roots concerned in the formation of this nerve were those of the 4th, 5th, 6th, and 7th.

Takahashi (331) noted atrophy of the testicle in the guinea pig after either abdominal sympathectomy or removal of the inferior mesenteric ganglion. This degeneration was limited chiefly to the seminal epithelium. Marconi (332) showed a similar atrophy in the testes of a dog under like conditions. Lipschütz and Vass (333) were unable to substantiate these findings in the cat. However, their experiments are not conclusive since they removed only the lowest two lumbar ganglia and this procedure does not denervate the genital organs. In the light of Bacq's (312) recent work this degeneration may be due to disuse, since such animals are able to copulate but are unable to eject semen.

Sensory nerve endings which are connected with nerve fibers in the pudic nerve were observed in this organ (330).

Epididymis. The same autonomic nerve branches supplying the testes also supply the epididymis (330, 334).

Vesicula seminalis or uterus masculinus. These two names are frequently used interchangeably by some investigators, but they are not really the same. The uterus masculinus is called the vesicula prostatica by Krause (222), uterus masculinus by Parker (224), vesicula vasis deferentis by Rauther (514) and Gerhardt (515), and vesicula seminalis by Wolhynski (336). According to Langley and Anderson (229, vol. xix) cats and rabbits do not possess seminal vesicles in the true sense, having in their stead an enlarged uterus masculinus which is regarded by anatomists as the homologue of the seminal vesicle. According to Schäfer (337) the uterus masculinus is the remains of the proximal end of the Müllerian duct, being a vestigial organ and the homologue of the uterus and vagina of the female.

The seminal vesicles in mammalia are described as being corrugated tubes having a thin wall composed of two layers of smooth muscle, an outer longitudinal and an inner circular (324, 338, 339). Enveloping the musculature is a fibrous coat containing many small ganglia (13, 16, 260, 336, 340, 341). According to Stöhr, in addition to the ganglia found in the adventitia, there are noted many nerve fibres and plexuses of nerve fibrils in the adventitia, muscularis, and submucosa.

The contour of the organ varies in different species of animals. In the guinea pig it consists of two long almost straight tubes which are thick at the proximal end, but slender at the distal end; in the rat each vesicle is short, thick and sacculated. The uterus masculinus in the rat is relatively large, projects behind the posterior poles of the prostate and fills the space behind the gland and the ampullae of the vasa deferentia. It opens into the floor of the urethra as in man and receives at that point the terminations of the seminal ducts.

Starling (342) believes the seminal vesicles go into peristaltic contractions during coitus but makes no reference to their behavior under other conditions than those accompanying sexual excitation.

It is agreed that the seminal vesicles are innervated by the hypogastric nerves (229, 296, 339), and are associated with the plexus deferentialis, hemorrhoidalis and vesicalis (260). These nerves originate from the 3rd, 4th, and 5th anterior lumbar roots in the rabbit and from the 2nd, 3rd, 4th, and 5th anterior roots in the cat. Some of these preganglionic fibres end in the inferior mesenteric ganglion, but most do not end until they reach the seminal vesicles, terminating there in local ganglia and joining very short post-ganglionic fibres. These results were shown upon painting the inferior mesenteric ganglia with nicotine and stimulating the sympathetic nerve fibres central to the ganglion and by cutting the nerve fibres and allowing them to degenerate before excitation of the nerve trunk. According to Langley and Anderson (229) the lumbar nerves cause in both rabbit and cat strong contraction of the whole musculature of the seminal vesicle, a view also held by Starling (322). Pallor independent of the contraction was also noted. The contraction of the seminal vesicle was strong enough to cause emission of semen from the penis. Unilateral stimulation of the nerve caused bilateral contraction of the seminal vesicles, beginning on the stimulated side. In no instance did stimulation of the sympathetic cause vaso-dilatation of the vessels in this organ.

The findings of these experimenters are supported by those of a number of others who observed ejaculation without erection of the penis,

upon stimulation of the spinal cord or upon electric stimulation of the pre- and post-ganglionic fibres of the autonomic nervous system innervating the genital organs (229, 310, 311, 312, 343, 344). Langley and Anderson could find no evidence that the seminal vesicles are innervated by the pelvic nerves, and they concluded that the sacral nerves send neither inhibitory nor motor fibres to any of the internal generative organs.

Stöhr, however, does not agree. He believes fibres from the 2nd, 3rd, and 4th sacral roots from the pelvic nerves supply it. Their function however, is not given.

The responses of this organ to sympathetic and para-sympathetic drugs do not confirm the physiological experiments in all details. Epinephrine causes contraction of the seminal vesicles in the rat and guinea pig (339, 345) and in the cat and rabbit (339, 57). The tone, rhythmical activity and the amplitude of contractions are increased by epinephrine. Although Waddell used ergot in his experiments, he made no mention as to the action of epinephrine following its administration.

Pilocarpine, when added to the bath in which the segment of seminal vesicle of rat or guinea pig is contracting, produces a moderate increase in tone accompanied by a marked increase in rate and force of the contractions in the spontaneously active organ, and initiates rhythmical contractions in a quiescent organ. Atropine antagonizes this effect. Waddell concluded that the positive reactions obtained with these drugs show the presence of para-sympathetic motor nerve fibres. Similar findings were recorded in the uterus masculinus in the rabbit (339).

THE SEMINAL DUCTS OR VAS DEFERENS. The vas deferens is the excretory duct of the testis and is a continuation of the epididymis. It begins at the globus minor and ascends along the back part of the testis on the inner side of the epididymis and passes with other structures in the spermatic cord into the pelvic cavity. At the base of the prostate it unites with the duct of the seminal vesicle to form the ejaculatory duct. The vas deferens consists of three coats, the outer of areolar fibrous tissue, the middle muscular and the inner mucous. The muscular coat consists mainly of two layers of unstriped muscle, the outer longitudinal and an inner circular, although some anatomists describe a third inner longitudinal layer, at the beginning of the duct (160) and other writers describe three continuous muscular coats (346).

In the adventitia is found a nerve plexus as well as many ganglion cells, especially in the region nearest the prostate gland (16, 330). Some authors have described fine nerve plexuses in the muscles as well as in the sub-mucosa (347), but this has been denied by others (16).

Although Nagel (348) reports a single stimulus as causing an isolated contraction of the vas deferens and Loeb (349) observed, upon hypogastric nerve stimulation in the rabbit, no peristaltic activity, Fick (350) noted true peristaltic contractions in the organ when it was stimulated electrically and Budge (215) was able to confirm this work. In addition, the last observer studied the effect of stimulation of the spinal cord at different levels and also the effect of stimulation of the hypogastric nerve upon the organ. In all cases the hypogastric nerve was found to be a motor nerve. The results from the stimulation of the spinal cord at different levels caused him to place the reflex centre for contraction of the vas deferens between the 4th and 5th lumbar vertebrae in the rabbit. Both Marshall (324) and Starling (342) believed that the vas deferens undergoes peristaltic contractions during coitus.

A detailed study of the origin of the nerve fibres supplying the organ was made by Sherrington (243), and Langley and Anderson (229). The former author found that stimulation of the anterior roots of the 12th thoracic, 1st, 2nd, and 3rd lumbar caused contraction of the vas deferens in the Rhesus monkey, and in all instances the 2nd and 3rd lumbar nerves gave a maximum contraction. In the cat he observed contraction of the organ upon stimulation of the 3rd and 4th lumbar roots. He believed these fibres are found outside of the spinal canal in the genital branch of the genito-crural nerve. Langley and Anderson were unable to find a fibre for the vas deferens in the genito-crural nerve and believe this report of Sherrington's was due "to a slip of the pen."

In rabbits and cats Langley and Anderson (229, vol. xix) noticed strong contraction and pallor of the vas deferens upon stimulation of the 2nd, 3rd, 4th, and 5th sympathetic lumbar nerve roots. The vas deferens, in contracting, shortened from one to three centimeters and they believed there could be no doubt that the contraction involved the longitudinal as well as the circular muscle coats. Similar views had been expressed by Schäfer (342) and by Langley (228) in an earlier research. The branches of the nerve described above run through the sympathetic to the inferior mesenteric ganglia whence they proceed by the hypogastric nerves to the vas deferens. Langley and Anderson pointed out the fact that the accessory hypogastric nerve does not send fibres to the vas but that only the main hypogastric nerve contributes fibres to this organ. In two cats Elliott (57) was unable to arouse the urinary bladder into activity by stimulating the hypogastric nerves, but in these the excitation caused a striking contraction of the vas deferens.

There seems little doubt that the sacral nerves (pelvic nerves) take no

part in the innervation of the vas deferens. Langley and Anderson were unable to show either motor or inhibitory nerve fibres to the vas present, in this region, upon varying the strength of electrical stimulation applied to the nerve roots.

Pharmacology. The physiological experiments find support in pharmacological ones only in the matter of the innervation of the organ by sympathetic nerve fibres. The failure to obtain contraction by electrical stimulation of the sacral roots is by no means confirmed pharamacologically. Waddell (346) was the first to study the effect of epinephrine on excised segments of vas deferens in sheep, dogs, rabbits, rats, and guinea pigs and in a later article he added cats to this list. In all instances epinephrine caused an increase in the general tonus and an increase in the force of contractions in rhythmically contracting organs, and in those which were not spontaneously rhythmical it induced rhythmicity after first increasing the general tone. These results were confirmed by Macht (345, 351), and by Macht and Cook (352) who added the organ of the opossum to the list.

That the sympathetic nerves contain inhibitory fibres as well as motor was reported by a number of workers (346, 345, 351, 353). In all of these ergotoxine caused an increase in general tone and in some instances increased rhythmicity. Following ergotoxine, the organ always responded to epinephrine by a loss in general tonus and in most instances by an inhibition of the contractions (346, 345).

The results obtained by the use of para-sympathico-mimetic drugs by the different authors agree in general (229). Waddell (346) and Macht (345), working independently, both showed that pilocarpine, and other so called para-sympathetic stimulatory drugs, increase the tone, rate and amplitude of contraction of the vas. In excised rats', rabbits', and cats' vas deferentia pilocarpine produced rhythmical contractions in quiescent organs. Atropine on the other hand caused immediate relaxation and quiescence.

Concerning these results, I should like to quote a few sentences from the authors themselves. Macht says "These findings show that the vas deferens is innervated by the sacral autonomic or para-sympathetic systems"—"As is well known certain drugs attack certain specific nerve endings and by the employment of such drugs, evidence as to the kind of innervation of those organs can be obtained which is as valuable as that derived from careful dissection and histological examination." And Waddell writes concerning the atropine effect—"This of course indicates paralysis of the para-sympathetic motor apparatus by atropine."

The experiments of Langley and Anderson may be added to support this view. They observed that atropine did not affect the sympathetic nerve endings. They injected huge doses of the drug, 50 mgm., intravenously, and did not observe any signs of decreased activity in the motor effect of stimulation of the peripheral end of the hypogastric nerve.

Gohara's (353) findings and those of Macht and Cook (352) are a little more difficult to fit into this scheme. The former experimenter observed atropine to cause an increase in general tonus contrary to what one would expect from the above reports and in these experiments a subsequent dose of epinephrine caused not contraction, as would be expected, but relaxation of the tone and disappearance of the contractions of the organ. He therefore argues that atropine depresses the motor sympathetics, an interpretation contrary to the findings of Langley and Anderson. Gohara believed epinephrine can cause either inhibition or contraction of the organ dependent upon the type of sympathetic stimulation, a fact already pointed out by other investigators. Although they made no mention of this fact, Macht and Cook published a record in which the tone of the vas, which had already been increased by the administration of physostigmine to the bath in which it was contracting, was decreased by the addition of epinephrine.

The explanation of these conflicting results is probably to be found in fact discussed on page 502. As stated there, we learn that if the tone is already low, epinephrine causes contraction, but if the tone is high, it causes a relaxation of the organ.

Although many workers (354, 320) explained a similar action of pilocarpine as being due to its stimulation of the sympathetic nerve endings, their theory does not seem to fit this case since an administration of atropine antagonizes the action of pilocarpine, rendering it impotent yet has no effect whatever on the effects produced by stimulation of the sympathetic nerve endings. In some other organs, also, atropine fails to paralyze the motor para-sympathetic mechanism to nerve stimulation.

CLITORIS AND LABIA MINORA. The clitoris is an erectile structure in the female analogous to the corpora carvernosa of the penis in the male. The labia minora forms the prepuce of the clitoris (160). As far as can be determined from the literature, the origin of the autonomic nerve supply to the clitoris and labia minor is the same as that of the penis in the male. Suffice it to say that it possesses efferent motor and vasoconstrictor sympathetic nerve fibres and efferent inhibitor and vaso-dilator fibres. These fibres are derived from the hypogastric and internal pudic nerves.

These two structures are of particular interest to the research student because of the development of sensory nerve endings and nerve plexuses (16, 334, 355–362, and others). Vater-Pacinian bodies which have been described by all investigators and which Ohmara (334) believes develop post-natally, reaching their full development at the time of puberty, are the sensory nerve endings most actively studied and of greatest interest.

Ovary. The ovary derives its innervation from the lumbar sympathetic through the ovarian plexus (plexus spermaticus) (16) or hypogastric plexus and from the sacral roots in the plexus sacralis (363, 364). The presence of ganglia of sympathetic origin in the hilus has been described by a number of men (364, 365–367). Others have described plexuses of nerve fibres about the blood vessels in the organ (252, 365, 366, 368). Still others have described what they call follicular nerves, i.e., nerves which penetrate the membrana granulosa and end in the Graafian follicle (366, 367, 369, 370), but confirmation of the last findings are denied by Retzius (371) and Mandl (368). Retzius studied the ovaries of cats of different ages. In these he never saw nerve fibres penetrate into the epithelium of the follicle and declares the positive findings to be artifacts.

In a later research he reported having noted that the follicular nerve fibres pass to the epithelium of the granulosa, but never into the follicle. These nerves form a fine network about the young follicles and a dense rich network around the adult follicle. He believes they innervate the smooth muscle stroma of the follicle and have no relation to its secretion.

His findings are supported by those of Lhermitte and Dupont (372) who noted the innervation of the ovary to be of little importance in reproduction and ovulation. Kraul (363) believes that the ovary is not only under the influence of the hormones but also under the influence of nerve impulses. He believes the parasympathetic nerves hasten the maturation of the follicle, since daily injections of pilocarpine in rabbits caused an acceleration of maturation, and that, on the other hand, the sympathetic nerves inhibit the maturation of the ovum since daily doses of epinephrine delayed its maturing. Hinsey and Markee (517) noted the fact that ovulation could be induced in female rabbits following the injection of human pregancy urine in the complete absence of the visceral afferent and efferent vagal pathways to the ovary, and of those nerve fibres which pass to and from the thoraco-lumbar and sacral segments of the spinal cord to the ovary. From their results the presence of intact nerve fibres to the ovary does not appear necessary for ovulation.

Investigators studying the innervation of the ovary have devoted most of their attention to the question as to whether or not the development of ovarian follicle is dependent upon its autonomic innervation. The vaso-motor nerves apparently have received very little study. Although Langley and Anderson (229) report in their introduction that they made observations on vasomotor changes in the testes and ovaries, a careful reading of their article reveals no reference to this point. In the frog Langley and Orbeli (373) were able to show that sympathetic stimulation (stimulation of the 4th, 5th, 6th and 7th spinal nerves) caused constriction of the blood vessels in the ovary.

FALLOPIAN TUBE. The fallopian tubes or oviducts convey the ova from the ovaries to the cavity of the uterus. They are made up of three coats, the outer serous, the middle muscular, in which the outer layer is longitudinally arranged and the inner circularly disposed, and the inner mucous. The greatest bulk of the organ is made up of smooth muscle which undergoes periodic rhythmical contractions.

The autonomic nerves of the fallopian tubes are received in part from the ovarian plexus and in part from Frankenhäuser plexus (utero-vaginalis) (16, 374–376). According to Langley and Anderson (229) the sympathetic fibres are derived from the 1st to the 5th lumbar roots in both rabbits and cats. Nearly all of these fibres proceed to the inferior mesenteric ganglion and thence to the organ by way of the hypogastric nerves. Kok (375) confirmed these observations.

The nerve fibres enter the adventitia and form there a dense network. Smaller fibres pass into the muscular layer where they form a very fine intramuscular nerve plexus (16, 360, 374, 367, 377–379). In some cases these fibres appear to penetrate the submucosa and even the mucosa itself (16, 374, 377). These nerve plexuses appear not only about the longitudinal and circular muscle cells, but they also surround the muscles of blood vessels as well (16, 374, 229). In all cases the efferent fibres apparently terminate with no special end structures, but as bare nerve endings (360).

The majority of experimenters (360, 16, 359) were unable to demonstrate ganglia in the tube itself. Roith (380) and Gawronsky (381) noted them to be present near the organ in the broad ligament (or mesosalpinx).

Sensory nerve end organs (Pacinian bodies) were observed in the tubes (382–383) and the fibres from these end organs pass to the cord by way of the last two thoracic and first lumbar roots (13, 374). These sensory organs are most abundant in the third of the tube nearest the fimbria

and Harting thinks they may be concerned with reflex production of peristaltic activity in the organ.

According to Langley and Anderson (229), stimulation of the lumbar nerves in rabbits and cats has two effects on the fallopian tube; it causes vaso-constriction, leading to pallor of the organ and it causes contraction of the whole musculature. The contraction involves both the longitudinal and circular layers. In the frog Langley and Orbeli (252, 373) observed contraction of the blood vessels in the oviduct, upon stimulation of either the sympathetic nerve trunk or its branches, 4th, 5th, 6th, and 7th spinal nerves. Elliott (57) also observed contraction of the fallopian tubes upon sympathetic stimulation.

Langley and Anderson could find no efferent fibres passing from the pelvic nerve (nervus erigens) to the fallopian tubes.

Pharmacology. The results with epinephrine support the theory of sympathetic motor innervation of the organ. Epinephrine causes contraction of the fallopian tube (57, 384). This contraction may take the form of an increase in tone alone or in conjunction with an increase in the rate and force of contraction. In some quiescent segments it initiates rhythmical responses. In the rabbit, Gohara (353) observed inhibition of the activity of the tube (decreased tone and decreased pendular movements) with small doses of epinephrine, but increased tone and activity with larger doses. In testing the action of the para-sympathetic stimulating drugs upon this organ, he found that atropine relaxed an organ, the tone of which had been increased by a dose of epinephrine, but contracted an organ to which no epinephrine had been added. It therefore, appears that in this organ epinephrine and atropine act antagonistically and atropine acts here upon the sympathetic nervous system. These findings are in keeping with those of Mislowsky and Bormann (326) who observed hypogastric nerve stimulation ineffective on the prostate gland following the injection of atropine. Gohara found pilocarpine ineffective.

Kok (375) working upon rabbits and cats came to an opposite conclusion. Although acetyl-choline had no effect upon a quiet tube, atropine caused relaxation of a contracting one. Epinephrine he found, contrary to the findings of Elliott (57), caused relaxation and cessation of activity in a previously active tube. He concluded, therefore, upon rather flimsy evidence, that the sympathetics relax the circular muscle and cause interruption of movement and that the sacral autonomics increase muscle tone and cause muscle contraction.

ROUND LIGAMENT. The round ligament of the uterus like the uterus

itself, undergoes spontaneous rhythmical contractions. In the monkey, Sherrington (243) saw an extremely short and striking contraction of the round ligament upon excitation of the 12th thoracic and the 1st, 2nd, and 3rd post-thoracic nerve roots. In 17 out of 27 cats he saw a similar response upon stimulating the 3rd and 4th lumbar roots. These nerve fibers he found passed into the genito-crural nerve and thence into the genital branch. The contraction was so quick that he could not believe it of non-striated muscle. Microscopic examination showed it to be striated muscle of ordinary appearance.

Rainey (385) in 1848, pointed out that the human round ligament consists largely of muscle tissue of the striated type.

Junkmann and Strass (386) observed the round ligament in the cat to behave as does the uterus. It has, they say, spontaneous tone and activity. This activity was not observed in the round ligament of the rabbit. Epinephrine inhibits the tone of this structure in the cat and causes tonus and rhythmical contractions to appear in the rabbit round ligament.

Ejaculatory duct. Since the ejaculatory ducts are merely the continuation of the vasa deferentia and are structurally the same, no separate discussion is needed in this report. Suffice it to say that the reaction of these is the same to autonomic nerve stimulation as is that of the vasa deferentia.

Vagina and uterus. Many investigators in discussing the autonomic innervation of these two organs discuss them as though they were one because they believe their reactions to nerve stimulation identical. That this is by no means true has been shown by numerous experimenters who have reported opposing actions in the two organs upon stimulation of the same nerves and upon the injection of the same chemicals in the common experimental animals. We will, therefore, discuss them separately in this review.

Vagina. The vagina extends from the vulva to the uterus. It consists of an internal mucous lining, a smooth muscular coat and, between these two layers, erectile tissue. The muscular coat consists of an outer longitudinal layer of muscle and an inner circular layer. The longitudinal fibres are continuous with the superficial muscular fibres of the uterus. The two layers of muscle are not distinctly separable from each other, but are connected by oblique fibres which pass from the one to the other (160).

Upon histological examination many fine bundles of nerve fibres are seen in the adventitia of the vagina which form a nerve plexus about the

vaginal wall. In the serosa of the upper two-thirds of the vagina a number of multipolar ganglion cells are distributed (359, 360, 380, 387–389), some isolated, others in groups. According to a number of investigators, fibrils from these ganglia pass to the muscular coat where an intramuscular plexus is formed. Other fibrils pass to the submucosa and build there a finer nerve plexus. It has also been reported that some of the fibrils pass from this plexus into the mucosa of the vagina proper (16, 358, 359, 377). Afferent end organs have been observed by Krause (222) and Kölliker (17). These have the structure of the Vater-Pacinian bodies.

Kehrer (390) studied the movements of the vagina in situ in laparotomized rabbits. He observed longitudinal and transverse contractions, peristaltic and anti-peristaltic in type, but at that time its automoticity was not deduced.

Other investigators believed the autonomic nerve fibres to the vagina can be influenced reflexly and that there exists in the lumbar spinal cord between the 5th and 7th lumbar vertebrae, a centre for rhythmic contraction of the organ (285, 388, 390). Another view commonly held by experimenters is that the vaginal musculature is automatically rhythmical, but that it can be influenced by reflex stimulation of sensory fibres in other parts of the body through this central mechanism (388, 393, 243, 391, 392).

The origin of the parasympathetic and sympathetic nerves of the vagina and their action upon it. In most cases, it is impossible to tell from his article whether a writer is speaking of the lower or upper ends of the organ. Since it is generally agreed that the two ends differ somewhat in their response to hypogastric and pelvic nerve stimulation, this lack of conciseness may be largely to blame for the failure of various experimenters to agree with one another. Fellner (394) observed contraction of the organ when the hypogastric nerves were stimulated but relaxation upon excitation of the pelvic nerves. The effect upon the rhythmical contraction of cutting certain roots to the vagina was studied by Kehrer (390) in 1864. Upon severing the sacral roots to the hypogastric plexus he noted that the rhythmical contractions cease. On the other hand, extirpation of the hypogastric plexus, cutting the internal spermatic nerve and the anterior uterine nerve did not influence its activity. He also observed contraction of the vagina upon electrical excitation of the sacral roots even in dead animals. The upper two-thirds of this organ are supplied by fibres from the plexus utero-vaginalis and the lower third by fibres from the plexus pudendus. Fibres are also contributed by the 2nd, 3rd, and 4th sacral nerves (16).

In the cat, Sherrington (243) noted that stimulation of the 8th and 9th sub-thoracic roots caused strong contraction of the orifice of the vagina. In the monkey he found this structure innervated by fibres from the 7th, 8th, and 9th sub-thoracic nerve roots. This action he observed to be bilateral but the tissue on the stimulated side always responded more strongly than that on the opposite side. When the orifice was cut in the median line, only homolateral responses were observed upon similar excitation. Gunn and Franklin (395) could find no clear differentiation in action between the vagina and the sphincter of the vagina to efferent nerve stimulation. In his early investigations, Langley (228) reported that the lower portion of the vagina in rabbits could be made to contract and occasionally to dilate on exciting any part of the sympathetic chain from the 2nd lumbar to the 4th sacral ganglion. As a rule the stimulation caused vaso-constriction but once in a while a slight vaso-dilatation of the mucous membrane was observed. After the injection of 10 mgm. of nicotine, stimulation in the lumbar region had no effect on the vagina, while stimulation of the sacral chain still caused contraction even after huge doses of nicotine had been given. He, therefore, concluded that the sympathetic fibres to the vagina were connected with nerve cells in the sacral sympathetic ganglia; and the fibres run from the ganglia up the gray rami communicantes to the sacral nerves, leaving only a few to pass by way of the hypogastric nerves. He observed that stimulation of the sacral nerves in the spinal canal also caused either contraction and pallor or dilatation (inhibition) and flushing of the vagina. Upon stimulation of these nerves dilatation and flushing were much more marked than the similar reaction to stimulation of the sympathetic nerves. Dilatation and flushing were the most noticeable effects seen upon stimulation of the 3rd and 4th sacral nerves and contraction and pallor that of the 1st and 2nd sacral nerves. Langley, however, believed that each sacral nerve could produce all possible effects. At the time of his early publications he believed the sacral nerve fibres to the lower part of the vagina were connected with nerve cells placed in or close to its walls. In a later article, Langley and Anderson (229) show that the vagina in cats and rabbits is innervated by efferent nerve fibres from the 2nd, 3rd, 4th, and 5th lumbar roots only, the sacral contributing no fibres to this organ. These fibres run by the white rami communicantes to the sympathetic, and nearly all of them run then to the inferior mesentric ganglia. From the inferior mesenteric ganglia they proceed to the hypogastric nerves. The efferent fibres are motor to the muscular wall and the effect is more constant in the rabbit than in the cat, though

in both it varied with the state of the organ with regard to parturition. Stimulation of these lumbar nerve roots caused slight contraction and pallor of the vagina. In no part of their course to the vagina do these nerves have any inhibitory or vaso-dilator action on the organ. Starling (342) reports similar results but limits the origin of the nerves to the 3rd, 4th, and 5th anterior roots.

That the vagina of the non-pregnant and pregnant cats may not respond in the same way to hypogastric nerve excitation was first pointed out by Kehrer (391) in 1907. In a nongravid cat he reported an effect similar to that of hypogastric excitation on the uterus, i.e., relaxation and inhibition of the organ. Inhibitory as well as motor fibres were believed to be present in the sympathetic nerve trunk by Falk (396). He based his opinion upon experiments on dogs and rabbits in which prolonged stimulation of the nerve caused relaxation of the organ following a period of contraction. This change he believed was due to a fatigue of the motor nerve fibres which permitted the action of the inhibitory mechanism to become visible. He noted that the parasympathetic system is motor in character.

The strongest supporters of the theory that the uterus and vagina do not behave alike when excited by electrical stimulation of the sympathetic nerves are Gunn and his collaborators (395, 397). As it is generally conceded that in the cat, guinea pig and rat the sympathetic nerve is inhibitory to the non-pregnant uterus, the question as to whether it is also inhibitory to the vagina was raised by them, since there is no other innervation to this organ. From earlier evidence they had come to the conclusion that the sympathetic nerve is not inhibitory to the vagina. They believed that during coitus, which occurs in the non-pregnant condition, contraction of the vagina occurs under nervous influence even though the only efferent nerve supply that the organ receives is through the sympathetic system. There appeared therefore grounds for supposing that in these, and indeed in all animals the sympathetic innervation of the vagina must be motor at least in the non-pregnant animal, regardless of whether it is motor or inhibitor in the uterus of the same animal when pregnant.

In every experiment upon excitation of the sympathetic in the rabbit, cat, rat, and guinea pig, they observed contraction of the vagina even though at the same moment the same excitation was causing in some cases relaxation and in others contraction of the uterus. This motor response to sympathetic stimulation or to epinephrine involving both longitudinal and circular muscle coats they found more marked in the half of the vagina nearest the vulva. See table 5.

Van Dyke (398) studied the effect of ergotoxin upon sympathetic stimulation of the vagina in cats. The normal responses he obtained,

TABLE 5

A table showing the responses of the gravid and non-gravid vaginas of experimental animals to hypogastric and pelvic nerve stimulation and to epinephrine

C contraction, R relaxation, C – R contraction followed by relaxation, 0 no response.

AUTHOR	YEAR	ANIMAL		VAGINA		
		Gravid	Non-gravid	Sacral	Sympathetic	Epinephrine
Fellner	1887			R	C	
Langley	1891			C or R	C or R	
Sherrington	1892		Cat	C		
			Monkey rhesus	C	C	
Langley and Anderson	1896		Cat	0	C	
			Rabbit	0	C	
Falk	1913		Dog	C	C – R	
			Rabbit	C	C – R	
Waddell	1917		Rabbit			C
			Dog			C
			Hog			C
			Sheep			C
			Cat			R
			Rat			R
			Cow			R
			Guinea pig			R
Gunn and Davis	1920		Cat			C
			Rat			C
			Guinea pig			C
Gunn and Franklin	1922		Cat			C
		Cat				C
			Rabbit			C
			Rat			C
			Guinea pig			C
Dale	1906	Cat			C	C
			Cat		R	R
			Rabbit		C	C
			Monkey			C

confirmed the findings of Gunn. However, after ergotoxin, stimulation of the hypogastric nerve no longer caused contraction but caused either a relaxation followed by contraction or a complete relaxation of the organ.

His findings also supported the inferences of Falk (396) who believed the hypogastric nerve contained motor as well as inhibitory fibres for this organ.

Pharmacology. There can be little doubt from the reports just presented that the sympathetic innervation of the vagina is motor in all animals, regardless of what its action upon the uterus is (395). That it also possesses an inhibitory sympathetic nerve supply cannot be denied (396, 398) and also that this inhibitory reaction may predominate over the motor effect in some animals (391). In addition to the sympathetic nerve supply, the vagina receives, according to some experimenters, a motor parasympathetic nerve supply (396, 398).

The results gathered from experimentation with certain drugs upon the vagina confirm the physiological and anatomical findings. Divergent results were obtained upon the same species of animals by different investigators using the same methods and the same chemical compounds. Waddell (399) employed longitudinal and transverse segments of the vagina from a number of species of animals. In all cases these sections of excised vagina showed spontaneous rhythmic contractions when suspended in oxygenated Tyrode's solution at body temperature. In the case of the vaginas of the rabbit, dog, hog, and sheep, the addition of epinephrine to the bath caused increased general tone and rate of contractions, but decreased amplitude at the tone climax. In the vaginas of cats, rats, cows, and guinea pigs, epinephrine caused marked depression in tone and rhythmicity in small doses and complete quiescence with subnormal tone in large doses.

These latter results were immediately contradicted by Gunn and Davies (397) and by Gunn and Franklin (395). In the isolated vaginal muscle of the non-gravid cat, rat, and guinea pig, Gunn and Davies observed contraction of the organ when it was under the influence of epinephrine even though the uterus relaxed. This response was the same whether isolated longitudinal or circular muscle segments were used. When a series of rings was employed the motor effects were greatest in those cut from that part nearest the vulvar orifice. Gunn and Franklin were able to show a similar response of the vagina in situ in cats, guinea pigs, and rats upon the intravenous injection of epinephrine.

That the sympathetic nerves contain inhibitory as well as motor fibres was demonstrated by Waddell (399). After paralyzing the motor sympathetic endings by the administration of ergotoxin he found that epinephrine caused relaxation of the vagina instead of contraction, thus confirming the observation of a number of other observers (228, 396, 398).

There is also some pharmacological evidence to the effect that the vagina receives parasympathetic nerve fibers although at present the results of these experiments are looked upon with considerable skepticism. Physostigmine and pilocarpine were seen by Waddell to increase the tone and amplitude of contractions of the rabbit's and rat's vaginas. Subsequent doses of atropine completely antagonized their effects. These findings with atropine do not agree with those of previous investigators (392, 388), who observed contraction of the vagina of cats, dogs, and rabbits upon the addition of this drug to the bath. Kehrer (391) working with dogs' and cats' vaginas noted three effects dependent upon the concentration of the drug; small doses of atropine increased the strength of contractions, moderate doses were followed by a rise in tone and then by a decline, and large doses either increased the tone and simultaneously decreased the amplitude of the contractions or caused relaxation of tone with a high amplitude of contraction.

In the light of the experiments of Langley and Anderson (229) which to my knowledge have not been repeated, it is evident that pilocarpine and atropine must act either upon the hypogastric nerve endings or upon the muscle directly. Both of these views have had supporters in the literature, but neither of them is founded upon firm experimental evidence. We believe it possible, however, to state at this time that the autonomic innervation of the vagina is certainly by way of the lumbar sympathetics which send both motor and inhibitor fibres to this organ and that the response is independent of the action upon the uterus. Whether the vagina is or is not innervated by the pelvic nerves has not been proved conclusively. Certainly much of the confusion in the literature is probably due to the loose usage of the term vagina, and no doubt also partly due to the condition of the tissue at the time of stimulation as well as due to the anesthesia employed.

Bartholin glands. According to Frankenhäuser, the Bartholin glands are innervated by the pudic nerve. Barrington (325) studied the secretion of mucin by the Bartholin glands in cats, guinea pigs, and rats. In cats he noted that stimulation of either the pelvic or hypogastric nerves caused secretion. This reaction occurred after a long latent period, and the activity of the gland continued for some time after cessation of stimulation. Excitation of the pudic nerves caused an instantaneous flow of mucin with practically no latent period but this flow ceased shortly though the stimulation was maintained. He believed this response was caused by the contraction of the smooth muscle within the gland itself, and he concluded that the Bartholin glands are inner-

vated by true secretory nerve fibres which have their origin in the autonomic nervous system.

THE UTERUS. In 1848, Tyler-Smith (400) described the uterus as the most important organ of the animal when he said, "It supports the race in the same way that the stomach and heart support the individual." Certainly no single organ in the body has been studied as thoroughly by experimenters with more conflicting results and opinions concerning its action and even its innervation. The great discrepancies in the results are not to be wondered at because results obtained in one set of experiments on a group of animals have been summarily assumed to be true of an entire species of animals, or even of all species of animals. Some of the factors which have no doubt greatly influenced the results and which have not been considered nor controlled and unified in the experiments are: condition of the animal at the time of experimentation, species of animal, anesthesia—kind and depth,—doses of drugs injected, rate of injection, excised and intact tissues, actual preparation of tissue, contact with foreign materials in the process of preparation, dimensions of strip used, exposure to varying degrees of temperature, duration of time between excision and use, chemical content of bath, pH of bath, temperature of bath and a host of others.

As early as 1865, Korner (401) warned against applying the results obtained with experiments performed on dead animals or excised tissues to those obtained on the intact tissues of normal animals, and Röhrig (402) repeated this warning when he stated that experiments performed upon excised tissue are not necessarily physiological. However, the results if properly obtained even though applicable only to a specific case do give considerable physiological information and also some clue as to the possible effects of chemical substances or nerve excitation, in the intact organ in the normal animal.

Roughly speaking the human uterus resembles a pear which is flattened anterio-posteriorly. It is retained in its position by the round and broad ligaments on each side. It consists of two unequal parts; an upper triangular portion, the corpus, and a lower cylindrical portion, the cervix. The entire posterior wall is covered by peritoneum. The organ itself is composed of three coats; an external or serous coat, a middle or muscular coat and an internal or mucous coat. The major part of the uterus is made up of bundles of non-striated muscle, which are united by a greater or lesser amount of connective tissue in which are found many elastic fibers. A great deal of work has been done upon the arrangement of the musculature of the organ. Bayer (403), Kreitzer (404) and Veit (405)

attempted to distinguish two layers in the human, while Ribemont-Dessaignes (406) was unable to make out definite layers of muscle bundles. All admit that the greater part of the uterine wall is made up of a mass of muscle which is perforated in all directions by blood vessels and in which it is impossible to make out any definite arrangement of muscle fibres. Da Costa (160) describes, in his text book of anatomy, three muscle layers, external, middle and internal. The external layer is a thin band of muscle tissue placed beneath the adventitia on the dorsal and ventral sides of the organ and is primarily connected with the ligaments of the uterus and fallopian tubes. The middle layer of fibres which is thickest, presents bundles of circular muscle fibres closely connected with blood vessels. The circular fibres about the internal os form a distinct sphincter. The internal or deeper layer consists of longitudinal fibres which are continuous with the superficial fibres. In experimental animals, cats, dogs, rabbits, guinea pigs, etc., a bicornate uterus is found (407). In these, two very definite layers of muscle can be detected, an outer circular and an inner longitudinal.

The uterus like other internal generative organs is supplied with a dense nerve plexus in its adventitia. Some of these fibres penetrate the muscular layer forming an intra-muscular network. Usually these fibres run parallel to the blood vessels and some penetrate smooth muscle cells of the blood vessels and uterus and form a fine fibrillar network (238, 408, 389, 16, 367). As far as can be determined these fibres end as bare nerve endings (369). Frankenhäuser (22) and Elischer (410) believed they traced the fibres into the muscle cells, and into the nucleus where they ended in the nucleolus. These observations are doubted by Stöhr (16) upon reasonable grounds we believe, since he could not find such fibres. He believed that since the old methods of staining were inferior to the newer ones and that the microscopic equipment used at that time was inferior to the modern microscope, the opinions of these earlier workers must have been based on artifacts.

Although a number of observers (411, 377, 412) described nerve fibres and even nerve plexuses in the submucosa and mucosa, Stöhr (16) was unsuccessful in his search for such nerve elements and therefore doubted their presence.

The belief is generally held that there is no foundation for the assumption of an intra-mural system of ganglia and that there are only cervical ganglia present in the region of the cervix uteri (16, 359, 401, 413–417). Other investigators (377, 389, 391, 408, 409) agree that there are cervical ganglia present but they think that there are others also. In the

uterus of the sheep and calf, Köstlin (377) describes ganglion cells in the mucosa, Kehrer (391) noted ganglion cells in the submucosa, Keiffer (389), Hoogkamer (408), and Gawronsky (381) described a complicated system of sympathetic ganglia and nerve trunks in the muscular wall of the uterus. These results have not been confirmed by more recent investigators (414, 359, 16) and therefore are probably erroneous.

Stöhr (16) noted a large number of ganglia present in Frankenhäuser's plexus or the plexus utero-vaginalis and usually also in the neighborhood of the cervix uteri. Occasionally instead of finding a number of ganglia bordering the cervix only one large ganglion, the cervical uterine, was found.

The cervical ganglia are at the level of the internal os, according to Gemmell (413) who described multiple ganglia posterior to the uterine artery and a little distance from the uterine wall. Both extra-mural and juxta-mural ganglia were described by Naiditsch (414). The extra-mural ganglia contain from 40 to 100 nerve cells each, while the juxta-mural ganglia rarely have more than 10 to 15 cells per ganglion. He never noted ganglion cells in the wall of the uterus or in the mucosa. Nerve fibres pass from these ganglia into the body of the uterus itself.

Influence of central nervous system. Harvey (418) observed peristalsis in the exposed uterus of a pregnant dog and maintained that the contractions of the uterus delivered the child "propria ab uteri vi." He therefore disagreed with Hippocrates that the child delivers itself by placing its feet against the fundus of the uterus and the head against the cervix and pushing, thus tearing the bladder and widening the vagina and vulva. Harvey's observations were confirmed by Venette (419) in the dog and supported by those of Graaf (420) in the rabbit.

Many observers found the uterine activity dependent upon impulses from the central nervous system inasmuch as stimulation of the cerebrum (5, 401, 421–427), the cerebellum and the medulla oblongata (5, 401, 428) and the spinal cord (422, 425, 426) caused increased tone and rate and amplitude of the contractions of the uterus. The existence of cerebral control of the uterus was acclaimed by Barbour and Copenhaver (429) after subjecting by a special method a part of the cerebral cortex and basal ganglia of a rabbit to the influence of cold and heat. In a pregnant animal, they observed the fact that cerebral cooling caused diminution in the volume of the uterine cavity with a tendency to increase the amplitude and the frequency of the contractions. Acconci (411) believed the central nervous system acted as a control to regulate the rhythm of the contractions. Falk (396) reported the fact that the cerebrum inhibited uterine contractions.

Other observers were impressed by the importance of the spinal cord in uterine contractions and were convinced that there was a centre in the lumbar region (5, 189, 308, 423, 430–433). Goltz and Freusberg, observed a female dog with her spine severed at the 1st lumbar vertebra pass through a period of heat, become pregnant and give birth to a living pup. A similar case was reported by Brachet (308). Friedenrich and Nasse (433) reported an instance of a young woman who, though she suffered a complete paralysis of the extremities, due to the fracturing of her 3rd and 4th cervical spines, gave birth to a dead fetus 2 days later.

Reflex centre. Perhaps the most important function of the cerebral spinal nervous system in respect to the uterus, is that it contains centres for reflex action. Any afferent nerve stimulation if sufficiently painful will cause contraction of the uterus (5, 396, 434, 435). Falk (396) has shown that stimulation of the central cut ends of the crural, sciatic or axillary nerves causes contraction of the uterus and a like change is caused by stimulation of the mammae (435), skin, serosa and mucosa of the stomach and intestine, and clitoris. It has also been demonstrated that these reflexes can be interrupted by destruction of the spinal cord from the 11th dorsal to the 4th sacral.

That the uterus can contract rhythmically independently of the central nervous system was shown by a number of men (182, 276, 411, 436–441).

Vagus nerve. A number of men (181, 256, 396, 421, 426) believed they were able to demonstrate motor nerve fibres for the uterus in the vagus nerve. Usually the experimental animals employed were rabbits, guinea pigs, and cats. In all cases stimulation of the distal end of the cut vagus caused contraction of the uterus. Kilian (421) was able to show that excitation of the central end of the cut vagus in the cat caused increased activity of the uterus. His findings were at once disproved by Spiegelberg (428), Frankenhäuser (22), and Obernier (431). In a more recent article Falk (396) observed strong contraction of the uterus upon stimulation of the peripheral cut end of the vagus. This is, however, not conclusive evidence that the vagus supplies the uterus with motor fibres because no effort was made to maintain a normal blood pressure during the experiment. Inasmuch as excitation of the peripheral stump of the vagus causes slowing of the heart and fall in blood pressure, the change in the uterus may have been due to an asphyxia thus produced.

Hypogastric nerve (nervi uterini). It has commonly been said that all the motor fibres reaching the uterus are derived from the sympathetic system (lumbar cord) and that the pelvic nerves (sacral roots) send no

branches to this organ. In the early literature both sympathetic and parasympathetic fibres were described as influencing the activity of the organ, but since the latter part of the last century, the sympathetic alone has been regarded by most investigators as being both motor and inhibitory. Within the last 25 years, sufficient evidence has accumulated to compel one to admit that the pelvic nerves may be influential in uterine activity. In 1772, Eustachius (442) taught that the sole motor innervation to the uterus was derived from the sympathetic nervous system and indeed, that portion of the sympathetic system derived from the lumbar ganglia. His findings receive support in the works of Longet (296) and Dahl (359). Reil (443) and Valentin (5) viewed the spermatic and hypogastric nerves as those supplying fibers to the uterus. The sympathetic nerve was thought by Scanzoni (444) to act merely as a regulator of uterine contractions. Since the writings of these earlier men a number of conflicting theories have appeared in the literature concerning the influence of the hypogastric nerve upon the uterus. Some of these opinions are: that it may have no effect upon the organ, (308, 421); it may inhibit the contractions of the longitudinal muscle from the body of the uterus to the vagina and the circular muscle in the cervix (394); it may be either motor or inhibitory in certain animals depending upon the condition of the organ (354, 242, 391); it may cause stimulation only (5, 425, 44, 426, 428, 401, 430, 22, 448, 390, 424, 229, 391, 396); it may be motor for both the circular muscle of the body of the uterus and vagina and longitudinal muscle of the cervix (394); it may be only sensory (445); it may be both sensory and motor (229, 390, 396), and finally it may be vaso-motor (229).

Although the best authorities (228, 229, vol. xix; 446, 245, 449, 380, 238, 290), establish the origin of the hypogastric nerve supplying the uterus in the 2nd, 3rd, 4th, and 5th lumbar nerve roots, minor differences of opinion exist concerning these branches. Langley (228) in his early work, believed most of the fibres for the uterus traversed the sympathetic in the region of the 4th to the 6th lumbar ganglia so that presumably most of them arose from the 3rd, 4th, and 5th lumbar nerves. In a later research done with Anderson (229), cats and rabbits being used as experimental animals, he came to the conclusion that the 2nd, 3rd, 4th, and 5th lumbar branches send fibres to the uterus and that occasionally a few fibres leave the sympathetic below the 6th lumbar ganglion apparently and run directly to the pelvic plexus. Medowar (238) like Langley and Anderson (229) occasionally found fibres from the 5th passing to the uterus. Gaskell (245) in his research traced the origin of these fibres to the last dorsal and the 1st and 2nd lumbar nerves.

Nowland (446) using a human cadaver was able to traced fibres from the 3rd and 4th lumbar ganglia only to the uterus. The fibres leaving these spinal roots run by the white rami communicantes to the sympathetic and nearly all of them run then to the inferior mesenteric ganglion (229). Gaskell states definitely that there are no cell stations in the inferior mesenteric ganglion for the autonomic nerve fibres to the uterus but that the fibres merely pass through it. This view is not held by Langley (228), who believes that most of the fibres which run to the inferior mesenteric ganglia are connected with nerve cells there. These fibres whether pre- or post-ganglionic in type pass from the inferior mesenteric ganglion and aortic plexus (438, 401, 22) to the pelvic plexus, the utero-vaginal plexus and to the cervical ganglia by way of the hypogastric nerves and then to the uterus proper. The accessory hypogastric does not supply the uterus. In the rabbit, stimulation of the strands to the inferior mesenteric ganglia has a variable effect. Most of the fibres run to the lower pair of inferior mesenteric ganglia and stimulation of these strands usually gives contraction of the uterus, but occasionally stimulation of the strands leading to the upper inferior mesenteric ganglia excites the uterus only and those fibres passing to the lower pair of ganglia influence the vagina only.

On stimulating the peripheral branches of the hypogastric nerve in the pelvic plexus separately, Langley and Anderson (229) obtained contraction and pallor of portions of the uterus on the same side, i.e., stimulating the three main vesico-uterine strands of the plexus, proceeding from the ventral to the dorsal strand, they observed separate contraction of the vagina, of the basal part of the uterus and of the terminal half to two-thirds of the uterus on the same side as the nerve stimulated. Anatomically, the strands of the pelvic plexus appear to proceed to a certain extent from the pelvic nerve. Be that as it may, Langley and Anderson (229) believe that the pelvic nerve does not carry efferent fibres to the uterus and the contraction of the uterus which is caused by stimulating the strands of the pelvic plexus can only be due to efferent hypogastric fibres.

In cats and rabbits, as observed by Langley and Anderson (229), the contraction of the uterus upon stimulation of the lumbar nerve fibres, involves all muscular layers. In favorable cases, the longitudinal shortening is most striking. When these nerves are stimulated on one side homolateral contraction is the rule though in favorable experiments, slight contralateral contraction is also visible. A number of experimeters agree with the above observers. v. Basch and Hoffman (434)

observed the fact that stimulation of the hypogastric nerves (nerves uniting the inferior mesenteric ganglion with the inferior hypogastric plexus) caused swelling of the cervix, while stimulation of the nervus erigens caused shortening of the body of the uterus. They concluded that these nerves act antagonistically, the one supplying the circular muscle layer, the other the longitudinal. Fellner (394), using the dog as experimental animal, came to the same conclusion, i.e., stimulation of the nervi erigentes caused relaxation of the cervix but contraction of the body while stimulation of the hypogastric nerve caused inhibition of the body. Whitehouse and Featherstone (447) presented evidence that the circular fibres of the uterus both in the corpus and cervix are stimulated by the sympathetic system and inhibited by the lumbar cord. The longitudinal fibres, on the other hand, both corporal and cervical and the longitudinal muscle of the vagina, in the case of the rabbit, are stimulated by the lumbar cord impulses and inhibited by the sympathetic nerve impulses. In reading their article one would imagine that they were speaking about the sympathetic system in both instances, since the the lumbar cord fibres are ordinarily regarded as sympathetic but at one point they insert the terms nervus erigens and the lumbar cord fibres as synonymous. It appears, therefore, that they believe the uterus receives fibres from both the sympathetic and parasympathetic systems and that the reactions to these nerves differ not only in the uterine muscle, but even in the layers of muscle.

Cushny (354) in 1906 stated that the sympathetic had a biphasic action. Upon stimulation of the hypogastric nerve, a powerful contraction of the whole uterus of the rabbit, whether virgin, pregnant or multiparous was noted. He believed the hypogastric nerve exercised no normal tonic influence on the uterus, either in virgin or pregnant animals, for its division caused no change except a quick contraction and then a return to the position of rest. Although the prevailing feature of the hypogastric action in the rabbit was contraction, the subsequent relaxation was sometimes followed by such marked dilatation and inhibition of the spontaneous movements that he believed that inhibitory fibres are also contained in the hypogastric nerve. In the virgin cat, Cushny (354), Dale (242), and Kehrer (391) observed excitation of the hypogastric nerve to cause relaxation only, and in pregnant cats, contraction. In two experiments on dogs, Langley (228) observed contraction followed by a well marked relaxation upon excitation of the nerve. Similar inhibitory effects were later noted by Dale and Laidlaw (450) on the uteri of virgin guinea pigs and cats.

To prove more definitely the presence of inhibitory fibres in the hypogastric nerve trunks, Cushny employed ergochrysin which supposedly paralyzes the motor sympathetic mechanism. After such administration, stimulation of the hypogastric nerve in rabbits and pregnant cats was followed either by a short contraction followed by a marked relaxation and inhibition of spontaneous movements or by pure relaxation and temporary disappearance of uterine activity. Similar findings were made by Dale upon stimulating the hypogastric nerve before and after doses of ergotoxin and upon the administration of epinephrine in place of hypogastric nerve stimulation.

Sacral autonomic (pelvic nerve, nervus erigens, nervi uterini sacralis). Since the publications of a series of articles by Langley and Anderson (229) in the early nineties in which they showed that stimulation of the pelvic nerves in the cat and rabbit is without effect on the uterus, inhibitory or motor, this organ has been regarded as being innervated by sympathetic nerve fibres only. They confirmed their physiological evidence that the uterus lacks a pelvic nerve supply by experiments on degeneration of nerve fibres after section of the nerve trunk. In the cat they cut the roots of the sacral nerves in the spinal canal and after five or more days examined the branches of the pelvic plexus for degenerated fibres. They believed these results completely vindicated their view that no efferent fibres pass from the sacral nerves to the uterus.

In the guinea pig Dale and Laidlaw (450) observed the uterus to be unaffected by stimulation of the pelvic nerves.

Before these works were published, the view that the sacral nerves send efferent fibres to the uterus was widely accepted and within the last 15 years experiments have been performed showing that perhaps this was not a mistaken idea after all. Ninety years ago Longet (296) presented evidence that fibres from the sacral nerves supplied the uterus and that they were motor in action. His observations have been confirmed by many others (22, 401, 402, 455, etc.).

Frankenhäuser (22) described a branch of the pelvic nerve of a rabbit arising from the 3rd sacral nerve, which he said passed to the uterine horns, though it gave off filaments to the bladder. This branch he called the nervus uterini sacralis and he stated that it passes to the ganglion uterinum. Langley and Anderson (229, vol. xx) in consequence of the description and drawing of this branch by Frankenhäuser, dissected the pelvic nerve of many female rabbits but never found such a branch. They, however, traced into the peripheral end of the pelvic (vesical) branch a bundle of fibres, which arose from one of the uterine

branches but found no degenerated fibres in the mesometrium following cutting of all the fibres. The ganglion uterinum they regarded as a part of the hypogastric nervous system.

Meyer and Gottlieb (453) do not agree with them. They say "although physiologists classify the uterus as possessing solely a sympathetic nervous control, it should be borne in mind that the nervus erigens carries along with its sympathetic fibres some nerve fibres which are pharmacologically parasympathetic." Lundberg (456) wrote in the same tone. He believed that both parasympathetic and sympathetic fibres go to the uterus but that the sympathetic is much stronger in effect and so conceals the weaker parasympathetic motor action.

The origin of the pelvic nerves for the uterus has been investigated a number of times since Langley's publication and a number of investigators believe that there is such an innervation. Gaskell in 1886 (245) believed some fibres from the 2nd and 3rd sacral roots pass to the uterus and are motor in function. Dahl (359) was able to trace fibres from the 4th and 5th sacral roots to the uterus. Ishikawa (457) showed the pelvic nerve for this organ to originate in the 4th and 5th sacral roots in the human but in the dog in the 2nd and 3rd roots. Nowland (446) dissected a human cadaver and noted that the sacral outflow from the 2nd, 3rd and occasionally the 4th roots make up the pelvic nerve (nervus erigens) which runs to the uterus. Stöhr (16) writes that the uterus is innervated by the hypogastric nerve and by the 1st, 2nd, 3rd, and 4th sacral pelvic nerves.

I shall report Ishikawa's experiments in some detail because of their importance in solving the problem of autonomic innervation of the uterus. He used experimental animals and was unable to confirm Langley and Anderson's observations. He noted Frankenhäuser's plexus to be a combinaton of the pelvic and hypogastric nerves. The "nervi pelvici" he found originated in man from the 4th and 5th sacral roots. In dogs, with 7 lumbar and 3 sacral vertebrae the pelvic nerve originates in the 2nd and 3rd sacral roots. He contended that because of anatomical differences in the spinal column, the 2nd and 3rd roots in the dog are the same as the 4th and 5th sacral roots in man. He performed two important sets of experiments. In the first he excised the uterus with its nerve supply and in the second the uterus was left intact but the right pelvic nerve was cut some distance from the plexus. Three weeks later the animals were killed and the entire lumbo-sacral cord excised and serially sectioned and studied. In these animals no changes in the anterior horn cells from the 1st to 7th lumbar were seen. At the level of

the first right sacral, degeneration of nerve cells was first seen. This was best seen in the 2nd and 3rd sacral roots, but some changes had occurred in the first sacral and last lumbar segments. The experiments in which total extirpation of the uterus was performed showed the same findings in the cord, except that even the 6th and 7th lumbar segments showed some changes. From these results he concluded that in dogs the uterus receives fibres from the 2nd and 3rd sacral nerve roots and perhaps from the 1st sacral and lower lumbar.

The above experiments are the first to be presented which directly contradict the work of Langley and Anderson (229) whose work has been the basis of the common teaching in physiology. The criticism which one has to offer concerning these results is that excision of the uterus and Frankenhäuser's plexus and cutting the pelvic nerve may very likely remove fibres passing to other organs instead of only those belonging to the organ under discussion.

Some observers think that the nerves are capable of influencing the circular and longitudinal muscle layers separately in addition to causing the purely motor stimulating action (308, 390, 396, 401, etc.). Brasch and Hoffmann (434), and Whitehouse and Featherstone (447) believe that the pelvic nerves cause contraction of the longitudinal and inhibition of the circular coats of the uterus while the hypogastric nerves cause contraction of the circular fibres and relaxation of the longitudinal ones. Fellner's (394) view differs slightly from this in that he believes the pelvic nerve causes relaxation of the cervix of the uterus and contraction of the body of the organ. According to Dyroff (454) the sympathetic nerves cause an increase in the tone of the musculature which involves both circular and longitudinal muscle layers. Stimulation of the parasympathetic on the other hand causes an increase in the peristaltic activity. If this hypothesis is correct, the two systems are not antagonistic to each other, but both systems are motor, the sympathetic predominating over the parasympathetic. That the pelvic nerves carry sensory fibres was noted by a number of observers (16, 229, 390, 396, 402, 437, etc.). Stöhr (16) believed afferent fibres from the uterus pass through the 12th thoracic and 1st lumbar and the 3rd and 4th posterior sacral roots, the former by way of the hypogastric nerves the latter group through the pelvic nerves. In a recent publication by Aburel (458) the utero-sacral nerve is treated as though exclusively sensory in action though less important than the utero-lumbar nerve, which he regarded as a mixed nerve.

Vasomotor action. It is generally agreed that the lumbar nerves have two effects on the uterus: contraction of the small arteries producing pal-

lor of the organ and contraction of the musculature (229, 242, 354, 359, 424). Lee (516) observed nerve fibres passing to the smallest blood vessels in the uterus. Langley and Anderson (229) were unable to show an inhibitory or a vaso-dilator action on the uterus no matter where the nerve was stimulated. These findings have been confirmed by Dale (242), Sugimoto (451), and Markee (452) who noted only vaso-constriction in the uterus following the injection of epinephrine. Following an injection of ergot both stimulation of the hypogastric nerve and epinephrine caused a distinct flushing of the uterus, according to Dale (242).

The pelvic nerves may contribute vaso-dilator nerve fibres to the uterus (359, 424, 453, 454). Markee (452) observed the vascularity of the uterus of the guinea pig, rabbit, and monkey to undergo cyclic variations that make it appear to blush and blanch. Both the speed and the extent of these vascular changes are affected by the time of the day and the stage of the oestrous cycle. About every minute a light area appeared at the upper end of the horn of the uterus and spread down toward the cervix and the whole uterus was white for only 10 to 15 seconds. This alternated with a red period of about 45 to 50 seconds' duration. The mechanism of these changes is not known but they are probably not due to the autonomic nerve impulses but are hormonal in origin.

Pharmacology. The results obtained by investigators upon the administration of sympatho-mimetic and parasympatho-mimetic drugs in the main parallel the findings of those who studied the effects of nerve stimulation. Such an agreement in response is to be expected by those who think that the uterus is innervated by parasympathetic nerve fibres. Ishikawa's (457) work appears to be the best evidence to substantiate this theory.

The drugs act upon both intact and excised uteri with practically the same results, as will be pointed out later, the ultimate response being largely dependent upon the degree of tonus in the muscle at the time of stimulation.

Epinephrine. Compilation of the results of a number of experiments by numerous investigators (see table 6) has established the fact that the effects produced by epinephrine on smooth muscle in various organs and in different species of animals are the same as those produced by excitation of the sympathetic nerves to these organs. According to Elliott (57) the evidence of this is so strong as to be undeniable. He believes that the converse is also true, namely, that if epinephrine produces a certain effect on involuntary muscle of an organ, that will be the effect or at least the predominant effect of stimulation of the sympathetic nerve supply to that organ.

TABLE 6

A table showing the responses of the uterus to epinephrine

P. pregnant, V. non-pregnant uterus, + contraction, +− contraction followed by relaxation, − relaxation of muscle tone, −+ relaxation of muscle followed by contraction, 0 no effect.

	P. RABBIT	V. RABBIT	P. DOG	V. DOG	P. CAT	V. CAT	P. GUINEA PIG	V. GUINEA PIG	P. HUMAN	V. HUMAN	MISCELLANEOUS ANIMALS
Cushny		+									
		+−			+	−					
Dale					+	−					+ Virgin monkey
Kehrer		+		−	+	−	+	+		+	
Fardon					+	−					
Barger and Dale					+	−					
Frohlich and Pick					+						
Kuroda		+		+	+	+					
Okomato		+−		−+	+	−+	+	−+			− V. rat
				−							
Quadiarullo					+	−	+	−			
Gunn and Davis						−		−			− V. rat
Zodenk					+	−					
Van Dyke and Gustavson					+	−					
Gunn and Franklin		+			+	−		−			− V. rat
Rosenblueth					+	−					
Kurdinowsky		+	+	+							
Biagi		+									
Bell and Hicks		+									
Falka and Flemming		+−									
Falk		+									
Gahara		+									
Gaddum	+	+									
Dyroff	+	+									
Suckow and Burget		0									
Nikoloeff		+									
M'Swiney and Brown	+	+					−	+			
Langley and Anderson	+	+									
Adler							−	−			− P. & V. mouse
Dale and Laidlaw							−				
Gunn and Gunn							−	−			+ P. & V. ferret
											− P. & V. rat
Sugimoto							+	−			
							−				
Cow							+				
Turolt							−	−	+	+	
Rubsamen and Kligermann										+	
Gunn										+	
Lieb									+	+	
Gruber									+	+	
Rucker									−		
Knaus and Clark											− P. & V. rat
Hazama											− V. rat
Tate and Clark											− V. rat
Seel											+ V. hog
Rudolph and Ivy			−	−							
Ivy, Hartman and Koff											+ − P. monkey

That epinephrine excited the rabbit uterus, excised or in situ, was first shown by Kurdinowsky (435) in 1904 and by Biagi (459) in 1905. Since then the fact has been confirmed repeatedly (table 6). The only author who disagrees with these findings is Okamoto (460) who found that large doses of epinephrine caused relaxation of the rabbit uterus. Nikolaeff (461) was unable to confirm this. In 1906 Cushny (354) and Kehrer (391), each working independently, showed that the functional state of the cat's uterus determines what its reaction to the injection of epinephrine and to sympathetic nerve stimulation is to be. In all cases the virgin uterus responded to epinephrine by relaxation, whereas the pregnant organ contracted. Similar responses of the guinea pig's uterus were reported by Kehrer (393), Dale and Laidlaw (450), Cow (462), and M'Swiney and Brown (463). The results on the guinea pig were confirmed by Adler (306) who found relaxation in both pregnant or non-pregnant organs and by Gunn and Gunn (464) who obtained relaxation in both guinea-pig and rat uteri. Sugimoto (451) attributed the contraction he observed in guinea pig uteri upon intravenous injection of epinephrine to ischemia and not to stimulation of the motor sympathetic mechanism.

As a rule the human uterus responds to epinephrine by increased tonus and in some cases by increased rate and force of contraction (465–469). This response is observed whether the uterus is pregnant or non-pregnant. However, if the uterine tissue is contracted at the time of the administration of the epinephrine, relaxation may result. Rucker (470) reports three cases in which epinephrine caused relaxation of a constriction ring in a pregnant uterus. In the non-pregnant monkey, Dale (242) noted contraction of the uterus following the injection of epinephrine. Ivy, et al. (471) noted a sudden contraction followed by prolonged relaxation and absence of rhythmical contractions in the pregnant monkey uterus subsequent to an injection of the drug.

On the dog's uterus the results following the administration of epinephrine are not constant. However, as hypogastric nerve stimulation does not give uniform results either, one could hardly expect more of epinephrine. Most observers noted contraction of the uterus following the intravenous injection of epinephrine or its addition to the bath in which an isolated segment was contracting. However, Rudolph and Ivy (472) noted inhibition of the movements of the uterus in both pregnant and non-pregnant animals following the intravenous injection of the drug. Similar findings had been previously reported by Kehrer (391) and Okamoto (460).

The mouse's uterus like the rat's uterus always responds to adrenalin by relaxation. Knaus and Clark (473) noted that epinephrine had a powerful depressant action on the rat's uterus either in situ or excised. In the last stages of pregnancy the inhibition was as powerful as that seen in a virgin rat. No difference in results could be detected between the uteri of rats in oestrus or dioestrus or between virgin and parous rats.

We believe, as previously pointed out, that the hypogastric nerves carry both inhibitory and motor fibres to the uterus (p. 502).

The fact that two conflicting sets of results are obtained by the use of epinephrine leads many men to believe that the state of tonus of the organ at the time of stimulation determines the reaction to be obtained (60, 67, 217, 463, 474, 475, etc.). Thus, if the tone is originally high, epinephrine causes relaxation but if the tone is low contraction occurs. This point will be further discussed later in this report.

Ergotoxin. The inhibitory fibres which normally predominate in the rat, mouse, virgin guinea-pig and virgin cat uteri can be demonstrated in the rabbit, gravid cat and guinea pig by the administration of ergotoxin. In general those uteri which previously responded to adrenalin by contraction respond by relaxation following the injection or addition of ergot.

Dale's (242) original work continues to remain the most important research on this question. He used chrysotoxin, after injections of which he obtained a perceptible inhibition of the rhythmic contractions in the rabbit's uterus, upon subsequent injections of epinephrine. In the cat's uterus whether gravid or non-gravid whatever the normal effect of epinephrine had been, inhibitor, motor or diphasic, Dale showed that the effect of another injection of epinephrine following an injection of ergot was pure inhibition of tone and rhythm. By this means he therefore demonstrated that the sympathetic nerve supply to the uterus is of mixed function and that the nerve may contain some fibres connected to purely inhibitory, others connected to purely motor myoneural junctions or, on the other hand, that the myoneural junctions themselves may be composite, consisting of both motor and inhibitor elements. He decided from results obtained with ergot that its active principle paralyzes the motor elements of that myotrophic structure or substance which is excited by epinephrine and by impulses in the fibres of the true sympathetic system, the inhibitor elements being relatively or absolutely unaffected. The question as to whether these so called myoneural junctions are morphologically differentiated or, as Langley suggested, distributed as "receptor substances" is not answered by Dale (242).

One thing is clear and that is that the structure or substance affected by epinephrine is also that which reacts to impulses in sympathetic nerve fibres, because the two kinds of motor stimuli suffer a similar extinction by ergot.

These results have been confirmed by Barger and Dale (300) using ergotoxin and by a large number of other investigators (354, 463, 464, 472, 477, etc.). Braun (476) found the time required to render the uterus insensitive to epinephrine by ergotoxin varied directly with the concentration a fact which would support the theory that there existed a chemical neutralization. Gaddum (478) studied the effects of ergotamine and epinephrine on the rabbit uterus. He observed that if the uterus became insensitive to a small dose of epinephrine after the administration of ergotamine, it would contract again if a more concentrated solution of epinephrine was used. He did not believe that there was a chemical combination of the two drugs, but advanced a theory that there is an area in the muscle on which epinephrine acts and that a fraction of this area is blocked by ergotamine so that in each case the concentration of epinephrine must be increased a certain amount to overcome the block. Tate and Clark (477) observed that the excitability of the uterus in virgin guinea pigs increased to such a degree when ergot was given that epinephrine caused a contraction instead of relaxation. Another explanation just as plausible would be that ergotoxin paralyzed the predominant inhibitory mechanism in the hypogastric nerves, leaving the motor mechanism intact.

That ergotoxin paralyzes the motor sympathetic mechanism in the urinary bladder is questioned by Macdonald and M'Crea (217). They believe the change in tone of the organ is the most important fact in its response to epinephrine. However, M'Swiney and Brown (463) noted no change in the tone or rate of rhythmic contractions in either rabbit or guinea pig uteri following the addition of ergotoxin to the bath. A subsequent addition of epinephrine, however, relaxed the muscle fibres.

Although experiments, which lend support to the theory that the response of the organ to epinephrine is influenced by the general tonus of the organ, have already been cited (page 502), a few on the uterus are added here. Using non-gravid rabbit uteri, Falta and Flemming (474) were able to show that epinephrine can cause either complete inhibition of contraction with loss of tonus or contraction, the response depending upon the tonus of the organ. They increased the general tonus of the uterus by exposing it to blood serum. When in this solution the uterus always relaxed upon the addition of epinephrine to the bath. When the

tone of the same segment was low, as when in Ringer's solution, the drug caused contraction.

M'Swiney and Brown (463) exposed the rabbit's uterus to pituitrin and to histamine both of which increased its tonus. In both instances, a subsequent dose of epinephrine caused relaxation. Cutting off the oxygen supply to the solution in which are uteri of rabbits and guinea pigs causes the former to relax and the latter to contract. The addition of epinephrine reverses the actions, the former contracting and the latter relaxing. The results with pituitrin have been confirmed by a number of men (471, 472, etc.).

Using Witte peptone to increase the general tonus of the uterus, Fröhlich and Pick (479) obtained the same general results.

The tone of the uterus may also be increased by the addition of pilocarpine, atropine, histamine, strophanthin, quinine, aloes, hydrastis, etc. In each case epinephrine antagonizes the action of the drug, whatever the point of supposed action of the drugs (306, 451, 456, 460, 480, etc.).

Questions difficult to answer are why uteri of certain species of animals always relax upon stimulation of the hypogastric nerves and by epinephrine while others always contract under similar conditions, and why pregnant uteri of the cat and usually the guinea pig respond to such stimulation in a different manner than do the non-pregnant organs.

A number of hypotheses have been offered in explanation but none of them is entirely satisfactory. Cushny (354) believed the muscle of the pregnant cat uterus more irritable and the change in the reaction between it and the virgin organ due to that fact. His hypothesis is supported by the findings of Knaus (481), and Obernier (430) but contradicted by those of Haddaeus (425), and Brown-Séquard (513). In a series of rabbits Knaus (481) sterilized one horn of the uterus, leaving the other with its embryo undisturbed. Segments were cut from the sterilized horn at different periods of gestation and its irritability to epinephrine tested. He observed it to become more irritable as the animal neared term. A somewhat similar explanation was given by Dale (242). He believed that during pregnancy there is a change in the nerve fibres so that the motor fibres predominate over the normally more powerful inhibitory ones. According to Fardon (482) there may be either a positive increase in the number of augmentory nerve fibres during pregnancy, or there may be a change in the muscle cells which renders them more responsive to impulses from motor fibres. A similar explanation is offered by Quadiarullo (483) who attributes the difference in action to

formation of new muscle in which the autonomic motor fibres predominate over the sympathetic inhibitory fibres. This, however, is not in accord with the observations of Sellheim (484). During pregnancy the pituitary gland undergoes definite hyperplasia (485–487) and some experimenters think that the increased secretion of pituitrin sensitizes the uterus to epinephrine and to impulses from the hypogastric nerves (451, 462, 464, 477). Although this action is denied by others (488) the bulk of evidence favors such an hypothesis.

Kehrer showed that repeated injections of pituitrin changed the inhibitor effect of epinephrine on the virgin cat's uterus to an excitor effect. Cow (462) in 1919 carried on an extensive study on this subject. He observed that if the uteri of cats or rats received a preliminary treatment of pituitrin, subsequent doses of epinephrine caused contraction in either pregnant or non-pregnant animals. In the latter animals this response is much more difficult to obtain. Similar results occurred with the uterus in situ. He found that the uteri of virgin cats and guinea pigs fed pituitary extract by mouth or given hypodermic injections over a period varying in length from 30 days to 18 weeks respond to epinephrine by contraction instead of relaxation. He therefore claims that there is no reason for believing the hypothesis that during pregnancy motor nerve endings in the uterus multiply to such an extent that they come to outnumber the inhibitor nerve endings.

This "pregnancy response" was also studied by Van Dyke and Gustavson (489), and Dyroff (454). The first observers injected lipiodal and aqueous extracts of mature corpora lutea into kittens. In these animals the uterus responded to hypogastric nerve stimulation and to epinephrine by contraction as in the pregnant animal, instead of by relaxation as in the virgin. That the development of muscle alone was not the cause of this response was shown by the fact that administration of placental and follicular liquid extracts produced marked hyperplasia of the uterus without causing the organs to respond by contraction to epinephrine.

Whether there is an actual increase in the number of muscle fibres or only an increase in the size of the fibres is also a debated question (16, 484).

Another theory advanced to explain the difference in responses in pregnant and non-pregnant uteri is that of the ratio of calcium and potassium. Generally speaking the two elements act antagonistically, i.e., if the calcium content of the Ringer's solution bathing the uterus is diminished, the result is relaxation; if the potassium content is decreased

the organ contracts (451, 473, 490, 493). Calcium seems to sensitize the sympathetic nerve endings and potassium seems to desensitize the same mechanism. Kehrer (494) studied the calcium content of the blood serum in normal and pregnant women. In pregnant women he found the calcium to vary from 7.02 to 7.53 mgm. per 100 cc. of blood. This decrease from the normal, 10.18 mgm., he attributed to the use of calcium in the formation of fetal bones. Near the end of pregnancy, the calcium content had returned to normal. This increase in calcium is thought by Blair Bell (495) to start labor. There are, however, many contradictory statements in the literature on the question which are impossible to discuss in this brief article. Zodenk (492) noted calcium to have the same effect as epinephrine on the pregnant and non-pregnant uterus of experimental animals. This fact would support the above hypothesis. He concludes that though reactions to the sympathetic nerve stimulation and calcium appear to be the same, calcium does not actually stimulate the sympathetic system. He found the actions of sodium and potassium ions and parasympathic stimulation the same.

Turolt (493) studied the effect of excess calcium and potassium ions in Ringer's solution used to bathe segments of the excised guinea-pig and human uteri. Increase in calcium caused increased general tonus in the guinea pig, but relaxation of the human uterus. Excess of potassium ions in the Ringer's bath caused relaxation of the guinea pig's uterus but contraction in the human. Epinephrine when added to the bath containing an excess of calcium caused further contraction of the gravid and non-gravid guinea pig's uterus, but relaxation of the non-gravid and contraction of the gravid human uterus. When added to the bath containing an excess of potassium, epinephrine caused further relaxation of the guinea-pig uterus and the gravid human organ, but contraction of the non-gravid human uterus. He believes the differences in action obtained with epinephrine described by the different observers due to a difference in the calcium-potassium concentration in the fluids used. Tate and Clark (477), unlike other investigators, observed an excess of potassium to produce contraction of the guinea-pig, rat, rabbit, and kitten uteri. Lack of potassium gave a similar result, whereas an excess of calcium caused relaxation of uteri of rats and guinea pigs, but contraction in those of rabbits and cats. In the kitten they observed the fact that calcium chloride could antagonize the inhibitory effect of epinephrine and that in the rat the inhibition of the uterus by epinephrine could be counteracted by potassium chloride. Bell and Hicks (496) found calcium chloride active only in pregnant and menstruating uteri.

The fact that such variable results are reported would tend to discount the theory that these ions have an effect in bringing about labor in pregnancy.

Pilocarpine and atropine. The greatest controversies in the literature on the autonomic innervation of the uterus have surrounded the actions of the parasympatho-mimetic drugs on this organ.

In the rabbit, pregnant cat (354, 391, 460), guinea-pig (450, 464), mouse (306), rat (460), and human (497) uterus, pilocarpine causes violent contractions of the organ. In all cases this effect can be arrested by the use of atropine, though during stimulation of the hypogastric nerves it continues to be effective. From these facts Cushny (498) in 1906, concluded that "pilocarpine and atropine appear to act on some structure which is independent of the hypogastric nerve." A few years later he (354) again investigated the action of pilocarpine in cats. In every case intravenous injection of pilocarpine nitrate was followed by the same changes as occurred from stimulation by epinephrine and of the hypogastric nerve. In the pregnant animal it caused contraction and in the virgin it caused relaxation of the organ. Pilocarpine, however, was found to differ from the others in that it is completely antagonized by small quantities of atropine, which usually have no effect on the reactions to the others. In addition, the pilocarpine action on the pregnant cat's uterus he found could be reversed by the administration of a large dose of ergotoxin. The fact that the action of pilocarpine on this organ is exactly the same as the action produced by hypogastric nerve stimulation suggested to him that the alkaloid probably affects some point on the path of the nervous impulses. Strong confirmation of this idea is afforded by the fact of the antagonism of large doses of ergotoxin to the motor effects of pilocarpine and of nerve stimulation. On the other hand, atropine which antagonizes pilocarpine completely, does not influence the passage of the nerve impulses nor the action of adrenalin. He also noted the fact that physostigmine caused contractions of the uterus which could be antagonized by atropine, but not by ergotoxin. None of the physio-chemical conceptions of these alkaloids explains all of these changes. Cushny's suggestion that there may be a set of receptors for epinephrine and ergotoxin lying in the nerve impulse path and a separate and independent receptor for atropine and pilocarpine associated with the impulse path but not on the direct course to the contractile substance would seem, as he says, an unusual explanation especially since the receptor path for atropine and pilocarpine in other organs appears to lie in the nerve impulse path.

Dale and Laidlaw (450) working on this problem found that in virgin guinea pig as in the virgin cat, the uterus is inhibited by excitation of the hypogastric nerve and by epinephrine. Pilocarpine, however, has a very intense motor action whether the horn is isolated and immersed in Ringer's solution or observed in situ and the alkaloid injected intravenously. They were able to confirm Cushny's observations on the inhibitor effect following the injection of the alkaloid in the non-pregnant cat's uterus under natural conditions, but were disappointed in the inconstancy of action. They observed a number of instances in which the uterus inhibited by epinephrine was stimulated to contraction by pilocarpine even in situ. Inasmuch as the uterus supposedly has no pelvic nerve supply, they attributed the action of pilocarpine on the uterus in the body to two effects; a direct motor action on the uterine muscle, and an indirect action stimulating the sympathetic nerve supply. The pilocarpine effect on the cat's uterus, though it resembles that produced by sympathetic nerve stimulation, is however, unlike that of epinephrine in that it does not survive excision of the organ. The excised virgin cat's uterus responds to pilocarpine by contraction instead of relaxation. Because of this Dale and Laidlaw (450) investigated further the effect of pilocarpine on other organs, especially upon the adrenal body, and they found that the adrenal output was doubled upon the injection of pilocarpine. If the adrenal glands were excised, pilocarpine injected intravenously did not relax the virgin cat's uterus. They decided therefore that the uterine inhibition produced in the non-pregnant cat uterus was due to increased secretion of epinephrine. That pilocarpine depletes the store of epinephrine in the gland was confirmed by Stewart and Rogoff (499) but counterdicted by Tscheboksoroff (500), Elliott (501), and Ehrmann (502).

Gunn and Gunn (464) also questioned this theory of Dale and Laidlaw (450). On two occasions they observed pilocarpine to cause relaxation of the rat's uterus and they noted the fact that this was antagonized by atropine. Inasmuch as they were dealing with excised tissues they believed epinephrine was entirely out of the picture and the action was purely a local one.

Cow (462) demonstrated increased sensitivity of the uterus to pilocarpine following repeated injections of pituitrin, a phenomenon similar to that observed with epinephrine. Whether this change in response to pilocarpine is due to increased sympathetic or parasympathetic irritability cannot be answered since v. Frankl-Hochwart and Fröhlich (488) showed that pituitrin increases the responsiveness of the bladder to stimuli from the nervus erigens.

That pilocarpine can produce either inhibition or excitation of the excised uterus was demonstrated by Kuyer and Wijsenbeek (503). In both guinea pigs' and cats' uteri they observed relaxation of the organ with small doses and contraction with larger doses. Seel (504) used segments of pig's uterus. Uteri of very young animals responded to pilocarpine by relaxation whereas segments taken from uteri of old animals responded by contraction. Another difference in reaction of the uterus to epinephrine and to pilocarpine is shown by the fact that pilocarpine is ineffective after histamine whereas adrenalin causes relaxation. After pituitrin both substances cause inhibition (479). Sugimoto (451) also noted that if the uterus was contracted by pilocarpine, it could be made to relax by epinephrine.

It is, perhaps, a good idea to mention here that pilocarpine has been employed clinically to contract the uterus in pregnancy, but this use has been discontinued because of the side actions (479).

The results with atropine are most discouraging. In general, if the uterus has responded to pilocarpine by contraction, atropine relaxes the organ. In the absence of pilocarpine, atropine has little or no effect (229, vol. xix, 354, 422). In some cases it decreases the tone and rate of contractions of the uterus (505) and in others, small doses increase the tonus of the uterus and large doses decrease it (306, 391, 451, 460). Peculiarly enough in those instances in which atropine increased the tonus this effect could be antagonized by epinephrine.

Perhaps the interesting results of Adler (306) and Gohara (353) should be mentioned here. Adler observed that if the uterine tone had been increased by atropine, it could be antagonized by the addition of epinephrine. Gohara (353) also demonstrated that if the tone of the uterus was increased by epinephrine this could be abolished by a subsequent addition of atropine. These drugs were, in these cases, mutually antagonistic.

Photographic registration of the action current was used in studying the rabbit uterus by Hasama (506). His method was similar to that employed by Bayliss and Bradford (507), upon studying salivary secretion. They found upon stimulation of the chorda tympani that the surface of the gland became negative, the hilus positive and the reverse was noted upon sympathetic stimulation. Upon stimulation of the nerves and the injection of drugs Hasama came to the conclusion that the uterus is innervated by both sympathetic and parasympathetic nerve fibres. The alterations in form of the electrogram of the uterus as result of sympatho-mimetic drugs is, he believes, specific, and in all

instances the tubal portion of the uterus become strongly negative to the vaginal portion, the amplitude of the contractions becomes larger and the frequency increased. With the parasympatho-mimetic drugs the negative waves become more frequent without increased general negativity of the uterus to the vagina. One of the drawbacks to such experiments is that the vagina is also responsive to these chemicals and that the observed difference in the action current between the two organs may be due merely to the difference in their response to the substance.

Another theory which has recently gained favor among physiologists in explanation of the action of sympathetic and parasympathetic stimulation upon organs is that of chemical or humoral stimuli. Its advocates believe that some chemical substances are discharged into the organ by the nerve impulses and that these chemical substances act upon the organ itself. At present only Henderson (518) has made such observations on the genito-urinary system. This investigator believes that acetyl-choline is set free in the muscle of the urinary bladder upon excitation of the pelvic nerves. This acetyl-choline is instrumental in producing the changes in the organ itself. Thus far his work has not been confirmed by other workers, nor noted in other organs of the genito-urinary system.

BIBLIOGRAPHY

(1) Langley: The autonomic system. 1922. Ergebn. d. Physiol., 1903, ii, 808; Journ. Physiol., 1911, xliii, 125.
(2) Gaskell: Autonomic nervous system, 1916, 63.
(3) Langley: Journ. Physiol., 1916, l, 225.
(4) Vesalius: De Corporis humans fabrica, Basil, 1559.
(5) Valentin: De functionibus nervi cerebri et sympathici, 1839, cl, 64. Repertorium f. Anatomie u. Physiol., 1841, vi, 327.
(6) Cushny: Secretion of urine. London, 1917, 8.
(7) Papin and Ambard: Journ. Urol., 1924, xi, 340.
(8) Pawlinko: Verhandl. d. Russ Chir. Puogoff Gesellschr., 1921.
(9) Bradford: Journ. Physiol., 1889, x, 390.
(10) Hirt: Zeitschr. f. Anat. Abt. 1, 1926, lxxviii, 260.
(11) Pappenheim: Arch. f. Anat. u. Physiol., 1841, 533.
(12) Hoebler: Zeitschr. f. Urol., 1922, xi, 377.
(13) Hryntschak: Zeitschr. f. Urol. Chirurg., 1925, xviii, 86; Pflüger's Arch., 1925, ccix, 542; Arb. a.d. Neurol. Inst. d. Wiener Univ., 1922, xxiv, 409.
(14) Schilf: Autonomic nervous system. Leipzig, 1926, 207.
(15) Maier: Arch. f. Path. Anat. u. Physiol., 1881, lxxxv, 49.
(16) Stöhr: Möllendorff's Handbuch der Mikroskop. Anat. d. Mensch., 1928, iv, 143; Zeitschr. f. Anat., 1926, lxxviii, 555.
(17) Kölliker: Gewebelehre, 1867, 5; Sitzungsber. Würzburg, 1893, 2; Würzburger Natur. Zeitschr., 1862, iii, 1.
(18) Retzius: Sitzungsb. d. Physiol. Med. Gesellschaft z. Würzburg, 1893, 1.

(19) Smirnow: Anat. Anzeiger, 1901, xix, 347.
(20) Disse: Bardeleben Handbuch d. Anat. d. Menschen, 1902, vii, 1.
(21) Kaufmann and Gottlieb: Amer. Journ. Physiol., 1931, xcvi, 40.
(22) Frankenhäuser: Die Nerven der gebärmutter undihre endigun in den glatten muskel fasern, Jena, 1867; Jenaische Zeitschr. f. Med. u. Natur., 1866, ii, 73.
(23) Heitzmann: Die Anatomie des Menschen, 1890.
(24) Arthaud and Butte: Arch. d. Physiol. norm. et Path., 1890, xxii, 379.
(25) Anten: Arch. int. d. Pharmacolodyn. et d. Therap., 1901, viii, 455.
(26) Nakazawa: Tohoku Journ. Exp. Med., 1924, v, 185.
(27) Spiro and Vogt: Ergebn. d. Physiol., 1902, i, 417.
(28) Masius: Bull. R. Ac. Belg. 1888, xvi, 62; Centrabl. f. Physiol., 1889, ii, 615.
(29) Schretzenmayr: Arch. f. Exp. Path. u. Pharm., 1931, clix, 545.
(30) Beco and Plumier: Arch. int. d. Physiol., 1906, iv, 265.
(31) Eckhardt: Beiträge zur Anat. u. Physiol., 1869, iv, 155.
(32) Goll: Cited by Eckhardt, loc. cit.
(33) Pearce: Amer. Journ. Physiol., 1914, xxxv, 151.
(34) Burton-Opitz: Pflüger's Arch., 1908, cxxiii, 533; ibid., 1908, xii, 221; ibid., 1909, cxxvii, 142; Amer. Journ. Physiol., 1916, xl, 437.
(35) Thompson: Journ. Physiol., 1894, xv, 433.
(36) Walti: Arch. f. Exp. Path. u. Pharm., 1895, xxxvi, 411.
(37) Lazzaro and Pitini: Maly's Jahrb. f. Their Chemie, 1899, xxix, 288.
(38) Eichelberg: Cited by Cushny: The secretion of urine. London, 1917, 186.
(39) Loewi: Arch. f. Exp. Path. u. Pharm., 1903, l, 326.
(40) Cow: Journ. Physiol., 1914, xlviii, 1.
(41) Bernard: Leçons de Physiol., exp., Paris, 1835; Leçons sur le Proprietes Physiol. des. liquides de'l'organisma, 1859.
(42) Knoll: Beitr. z. Anat. u. Physiol., 1872, vi, 41.
(43) Klecki: Arch. f. Exp. Path. u. Pharm., 1897, xxxix, 173.
(44) Vogt: Arch. f. Anat. u. Physiol., Physiol. Abt., 1898, 399; Ergebn. d. Physiol., 1902, i, 417.
(45) Grek: Arch. f. Exp. Path. u. Pharm., 1912, lxviii, 305.
(46) Rhode and Ellinger: Zentralb. f. Physiol., 1913, xxvii, 12.
(47) Jungmann and Meyer: Arch. f. Exp. Path. u. Pharm., 1913, lxxiii, 48.
(48) Gubergritz: Zeitschr. f. d. ges. Exp. Med., 1926, lii, 619.
(49) Cohnheim and Roy: Arch. f. Path. u. Physiol., 1883, xcii, 424.
(50) Bayliss and Fee: Journ. Physiol., 1930, lxix, 135.
(51) Renner: Deutsch. Arch. f. klin. Med., 1913, cx, 101.
(52) Milliken and Karr: Journ. Urol., 1925, xiii, 1.
(53) Tournade and Hermann: Compt. rend. soc. d. biol., 1926, xciv, 656.
(54) Biedl: Pflüger's Arch., 1897, lxvii, 443.
(55) Block: Sperimentale, Firenze, 1925, lxxix, 895.
(56) Bunch: Journ. Physiol., 1897–98, xxii, 365.
(57) Elliott: Journ. Physiol., 1905, xxxii, 401; ibid., 1906, xxxv, 367.
(58) Cannon and Lyman: Amer. Journ. Physiol., 1913, xxxi, 373.
(59) Spadolini: Arch. d. fisiol., 1917, xv, 229; ibid., 1918, xvi, 135, 151.
(60) Gruber: Amer. Journ. Physiol., 1918, xlv, 302.
(61) Dale and Richards: Journ. Physiol., 1919, lii, 110.

(62) Kolm and Pick: Pflüger's Arch., 1920, clxxxiv, 79.
(63) Thomas and Wheelon: Journ. Lab. Clin. Med., 1922, vii, 375.
(64) Hirt: Zeitschr. f. Anat., Abt. 1, 1924, lxxiii, 621; ibid., 1930, xci, 581.
(65) Carlson, Boyd and Pearcy: Amer. Journ. Physiol., 1922, lxi, 14.
(66) Carlson and Litt: Arch. Int. Med., 1924, xxxiii, 281.
(67) M'Crea and M'Swiney: Journ. Physiol., 1926, lxi, 28; Quart. Journ. Exp. Physiol., 1928, xviii, 301.
M'Crea, M'Swiney and Stofford: Journ. Physiol., 1925, xv, 201.
M'Crea and MacDonald: Quart. Journ. Exp. Physiol., 1928, xix, 161.
Brown and M'Swiney: Quart. Journ. Exp. Physiol., 1926, xvi, 9; Journ. Physiol., 1926, lxi, 261; ibid., 1930, lxx, 253.
(68) Sharpey-Schafer: Journ. Physiol., 1931, lxxi, 362.
(69) Schwartz: Arch. f. Exp. Path. u. Pharm., 1900, xliii, 13.
(70) Barcroft and Brodie: Journ. Physiol., 1905, xxii, 18.
(71) Asher et al.: Zeitschr. f. Biol., 1906, xlvii, 1; ibid., 1913, lxiii, 83; ibid., 1916, lxiv, 441; ibid., 1917, lxvii, 327; ibid., 1917, lxviii, 31; ibid., 1914, lxiv, 441.
(72) Pearce and Carter: Amer. Journ. Physiol., 1915, xxxviii, 350.
(73) Mauerhofer: Zeitschr. f. Biol., 1917, lxviii, 51.
(74) Benedict and Nash: Journ. Biol. Chem., 1921, lxviii, 463; ibid., 1922, li, 183.
(75) Holmes and Patey: Biochem. Journ., 1930, xxiv, 1564.
(76) Ellinger: Arch. f. Exp. Path. u. Pharm., 1921, xc, 77.
(77) Ellinger and Hirt: Arch. f. Exp. Path. u. Pharm., 1925, cvi, 135.
(78) Hara: Zeitschr. f. Biol., 1922, lxxv, 179.
(79) Kichikawa: Biochem. Zeitschr., 1925, clxvi, 362.
(80) Kusakari: Tohoku Journ. Med., 1930, xvi, 509.
(81) Lobenhoffer. Mitteil. z. Grensgeb. d. i. Med. u. Chirurg., 1913, xxvi, 197.
(82) Legum and Flandrin: Presse Méd., 1923, xxxi, 741.
(83) Koennecke: Zeitschr. f. Urol. Chirurg., 1923, xiii, 157.
(84) Ambard: Atti del 2 Congresso intern. di Urologia, Roma, 1924,
(85) Seres: cited by Lurz: Deutsch. Zeitschr. f. Chir., 1925, cxciv, 42.
(86) Meltzer: Arch. klin. Chir., 1925, cxxvi, 127.
(87) Lurz: Deutsch. Zeitschr. f. Chir., 1926, cxciv, 42.
(88) Nisio: Arch. ital. Chir., 1927, xvii, 577.
(89) Gironcali: Zeitschr. f. Urol. Chir., 1929, xxvii, 266.
(90) Bellido: Treb. de la Soc. biol. Chir., 1923, cxxvi, 74.
(91) Boeminghaus: Arch. f. klin. Chir., 1923, cxxvi, 74.
(92) Caputi: Arch. di farmacol. Sper., 1929, xlvi, 210.
(93) Carrel and Guthrie: Science, 1906, xxiii, 394.
(94) Zaaijer: Beitr. z. klin. Chir., 1914, xciii, 223.
(95) Quinby: Journ. Exp. Med., 1916, xxiii, 535; Amer. Journ. Physiol., 1917, xlii, 593.
(96) Dederer: Journ. Amer. Med. Assoc., 1918, lxx, 6; Surg., Gynec., Obstet., 1920, xxxi, 45.
(97) Williams: Journ. Urol., 1926, xvi, 231.
(98) Hess: Journ. Urol., 1926, xvi, 191.

(99) Paunz: Zeitschr. f. d. ges. exp. Med., 1930, lxxi, 688.
(100) Marshall and Kolls: Amer. Journ. Physiol., 1919–20, xlix, 302.
(101) Yagi and Kuroda: Journ. Physiol., 1915, xlix, 162.
(102) Yoshimura: Tohoku Journ. Exp. Med., 1920, i, 113.
(103) Marshall and Crane: Amer. Journ. Physiol., 1922, lxii, 330.
(104) White and Martin: Genito-urinary surgery and venereal diseases, 1920, xii, 589.
(105) Howell: Textbook of physiology, 1918, vii, 863.
(106) Israel: Deutsch. Med. Wochenschr., 1888, cited by Barbey: Zeitschr. f. Urol. Chir., 1913, i, 567.
(107) Wertheimer: Arch. d. Physiol., 1894, xxvi, 724.
(108) Délezenne: Arch. d. Physiol., 1894, xxvi, 446; cited by Spiro-Vogt: Ergebn. d. Physiol., 1902, i, 419.
(109) Horder: Deutsch. Med. Wochenschr., 1911, 10.
(110) Macht: Journ. Pharm. Exp. Therap., 1926, xxvii, 329.
(111) Schönbauer and Whitaker: Wien. Klin. Wochenschr., 1925, xxxviii, 580.
(112) Blatt: Arch. f. Exper. Path. u. Pharm., 1930, cliii, 67.
(113) Langendorff: Zentralb. f. Physiol., 1908, xxi, 551.
(114) Langley: Journ. Physiol., 1901, xxvii, 237.
(115) Bardier and Frenkel: Compt. rend. Soc. biol., 1899, li, 544.
(116) Biberfeld: Pflüger's Arch., 1907, cxix, 341.
(117) Ogawa: Arch. f. Exp. Path. u. Pharm., 1912, lxvii, 89.
(118) Jonescu: Wien. klin. Wochenschr., 1908, xxi, 513.
(119) Marshall and Davis: Journ. Pharm. Exp. Therap., 1916, viii, 525.
(120) Kusakari and Takeda: Tohoku Journ. Exp. Med., 1930, xvi, 494.
(121) Arnstein and Redlich: Arch. f. Exp. Path. u. Pharm., 1923, xcvii, 15.
(122) v. Mering: Zentralb. f. inn. Med., 1886, vii, Beil. pp. 24, 29; ibid., 1887, viii, Beil. pp. 56 and 65.
(123) Zuntz: Arch. f. Anat. u. Physiol., Physiol. Abt., 1895, 570.
(124) Lépine und Klemperer: Berliner Verein. f. inn. Med., 1896, xv, 6.
(125) Baer and Blum: Deutsch. Med. Wochenschr., 1908, xxxvi, 1543; Arch. f. Exp. Path. u. Pharm., 1911, lxv, 1.
(126) Bélak: Biochem. Zeitschr., 1912, xliv, 291.
(127) Frank: Therapie d. gegenw., 1914, xxiv, 209; Verhandl. d. Deutsch. Gesell. f. inn. Med., 1921, xxxiii, 240.
(128) Grote: Verhandl. d. Deutsch. Gesell. f. inn. Med. 1921, xxxiii, 291.
(129) Eisner and Forster: Berlin. klin. Wochenschr., 1921, lviii, 839.
(130) Jöel: Therap. d. genenw., 1922, lxiii, 323.
(131) Koppányi: Klin. Wochenschr., 1925, viii, 361.
(132) Kokkalis: Deutsch. Zeitschr. f. Chir., 1930, ccxxix, 129.
(133) Teschendorf: Klin. Wochenschr., 1924, xl, 1811.
(134) Grunke: Zeitschr. f. d. ges exp. Med., 1926, lii, 488.
(135) Hetényi and Pogány: Verhandl. d. Deutsch. Gesell. f. inn. Med., 1926, 306.
(136) Kemper: Arch. f. Exp. Path. u. Pharm., 1927, cxxii, 73.
(137) Hetényi und Patai: Zeitschr. f. Exp. Path. u. Pharm., 1927, cxxii, 73.
(138) Goldberg: Zeitschr. f. d. ges. exper. Med., 1928, lix, 468.
(139) Dünner: Therap. d. gegenw., 1922, lxiii, 321.

(140) Bertram: Münch. Med. Wohenschr., 1925, xix, 772.
(141) Dünner and Mechlenburg: Zeitschr. f. d. ges exper. Med., 1926, li, 513.
(142) Ozaki: Acta Scholae Med., Kioto, 1917, ii, 143.
(143) Zamaguchi: Nisshin Igaku, 1922, iv, 997.
(144) Nishimaru: Reviewed by Nahazawa: Tohoku Journ. Exp. Med., 1924, v, 185.
(145) Hamet: Compt. rend. soc. d. biol., 1926, xciv, 727.
(146) Muschat: Journ. Urol., 1926, xvi, 351; Amer. Journ. Med. Sci., 1928, clxxvi, 851; Journ. Pharm. Exp. Therap., 1929, xxxvii, 297; Journ. Lab. Clin. Med., 1929, xiv, 364.
(157) Kümmel: Arch. f. klin. Chir., 1919, cxii, 1050; Zentral. f. Chir., 1922, 616; Zeitschr. f. Urol., 1924, lxxxv, 49; Klin. Wochenschr., 1925, 439.
(148) Casper: Referat. a. d. Urologenkong., Berlin, 1924.
(149) Schneider: Zeitschr. f. Urol., 1925, xix, 700.
(150) Eppinger: Therap. Halbmh., 1921, 126.
(151) Haebler: Zeitschr. f. Urol., 1925, xix, 332.
(152) Volhard: Klin. Wochenschr., 1925, iv, 145.
(153) Valdoni: Zeitschr. f. Urol., Chir., 1929, xxvi, 50; Policlinico, sez Chir., 1928, xxxv, 373.
(154) Stöhr: Zeitschr. Anat. 1921, liv, 529; ibid., 1924, lxxi, 313.
(155) Lehmann: Zeitschr. f. Urol., 1926, xx, 167.
(156) Fischer: Deutsch. Zeitschr. f. chir., 1930, ccxxii, 228.
(157) Müller: Die Lebennerven, 1924, ii, 1.
(158) Kappis: Zentralbl. f. inn. Med., 1925, xlvi, pp. 941 and 1097.
(159) Gubergritz and Istschenko: Zeitschr. f. Exp. Med., 1926, lii, 619.
(160) DaCosta: Gray's Anatomy, 1906, 1426.
(161) Piersol: Human anatomy, 1907, 1895.
(162) Quain: Elements of anatomy, 1898, iii, iv, 204.
(163) Young: Practice of urology., 1926, i, 1.
(164) Langley and Anderson: Journ. Physiol., 1895, xix, 71.
(165) Langley: Schaefer's Textbook of physiology, 1898, ii, 616.
(166) Engelman: Pflüger's Arch., 1869, ii, 243.
(167) Disselhorst: Anatomische Hefte, 1894, iv, 137.
(168) Lustig: Berichte der Kaiserl. Akadamie zu Wein, 1881, lxxxiii, 3.
(169) Wolff: Arch. f. Microskop. Anat., 1882, xx, 361.
(170) Haebler: Zeitschr. f. Urol., 1922, xvi, 377; ibid., 1925, xix, 332.
(171) Obersteiner: Strickler's Handbuch der Lehre von dem geweben, etc., 1871.
(172) Dogiel: Arch. f. Mikr. Anat., 1878, xv, 65.
(173) Protopopow: Pflüger's Arch., 1897, lxvi, 1.
(174) Alksne: Folia Urolgica, 1907–08, i, 338.
(175) Primbs: Zeitschr. f. Urol., Chir., 1913, i, 600.
(176) Satani: Journ. Urol., 1919, iii, 274; Amer. Journ. Physiol., 1919, xlix, 474; ibid., 1919–20, l, 342.
(177) Boeminghaus: Deutsch. Zeitschr. f. Chir., 1923, clxxix, 129; Zeitschr. f. Urol. Chir., 1924, xiv, 71.
(178) Macht: Journ. Pharm. Exp. Therap., 1916, viii, 155, 262; ibid., 1919, xii, 255; ibid., 1926, xxvii, 389; Journ. Urol., 1917, i, 97.

(179) LANGLEY: Journ. Physiol., 1913, xlvii, 159; ibid., 1914, xlviii, 73; ibid., 1915, xlix, 322.
(180) EDMUNDS AND ROTH: Amer. Journ. Physiol., 1908, xxiii, 28.
(181) GASSER: Journ. Pharm. Exp. Therap., 1926, xxvii, 395.
(182) VAN ESVELD: Holländ. Diss. Utrecht., 1927; Arch. f. Exp. Path. u. Pharm., 1926, cxix, 62; ibid., 1928, cxxviii, 116; ibid., 1928, cxxxiv, 347; Zeitschr. f. Mikros. Anat. Forsch., 1928, xv, 1.
(183) LAWRENTJEW: Internal Monatschr. f. Anat., u. Physiol., 1914, xxx, 337; Zeitschr. f. Mikros. Anat. Forsch., 1926, vi, 467.
(184) TIEGS: Australian Journ. Exp. Biol. Med. Sci., 1924, i, 131; ibid., 1925, ii, 157.
(185) VOLTERRA: Zentralb. f. inn. Med., 1925, xlvi, 876.
(186) GRUBER: Journ. Urol., 1928, xx, 27; Journ. Pharm. Exp. Therap., 1930 xxxix, 449.
(187) MULDER: Nederl. Lancet, 1845–46, 611.
(188) DONDERS: Physiol. d. Mensch., 1857, i, 498.
(189) MAYER: Hermann's Handb. d. Physiol., 1883, v, 1, 456.
(190) SOKOLOFF AND LUCHSINGER: Pflüger's Arch., 1881, xxvi, 464.
(191) STERN: Thèse de geneva, 1903.
(192) LUCAS: Amer. Med., 1905, ix, 744; Med. News, 1905, lxxxvii, 87; Science, 1905, xxi, 721; Proc. Soc. Exp. Biol. Med., 1908, v, 44; Amer. Journ. Physiol., 1906, xvii, 392; ibid., 1908, xxii, 245.
(193) LUDWIG: Wagner's Handb., 1844, ii, 639.
(194) GRAVES AND DAVIDOFF: Journ. Urol., 1925, xiv, 1; ibid., 1923, x, 184.
(195) BUDGE: Untersuch. u. d. Nervensystem; 1841 i, 159; cited by PFLAUMER: Zeitschr. f. Urol., 1919, xxiii, 367, 407.
(196) ROTH: Amer. Journ. Physiol., 1917, xliv, 275.
(197) LANGLEY AND ANDERSON: Journ. Physiol., 1926, xx, 376.
(198) CAPORALE: Journ. d'Urol., 1929, xxviii, 28.
(199) FAGGE: Journ. Physiol., 1902, xxviii, 304.
(200) HENDERSON: Journ. Physiol., 1905–06, xxxiii, 175.
(201) GIBBS: Amer. Journ. Physiol., 1929, lxxxvii, 594.
(202) HEPBURN: Surg., Gynec. and Obstet., 1919, xxviii, 316; New England Journ. Med., 1931, cciv, 1.
(203) WHARTON AND HUGHSON: Journ. Urol., 1931, xxv, 145.
(204) STEWART AND BARBER: Ann. Surg., 1914, lx, 723.
(205) BRODIE AND DIXON: Journ. Physiol., 1904, xxx, 492.
(206) PENFIELD: Amer. Journ. Med. Sci., 1921, clx, 36.
(207) PENTIMALLI: Sperimentale Arch. d. Biol., 1924, lxxviii, 457; reviewed in Physiol. Abst., 1925, x, 529.
(208) BAULET: Compt. rend. d. l. soc. d. biol., 1913, lxxiv, 1171; ibid., 1914, lxxvii, 355.
(209) EDMUNDS AND ROTH: Journ. Pharm. Exp. Therap., 1920, xv, 189.
(210) CUNNINGHAM: Textbook of anatomy, 1906, 1156.
(211) GRIFFITHS: Journ. Anat. Physiol., 1894, xxviii, 107; ibid., 1895, xxix, 61, 254.
(212) WESSEN: Journ. Urol., 1920, iv, 279.
(213) KALISCHER: Die Urogenitalmusculature des Dammes. Berlin, 1900.

(214) GRUBER: Journ. Urol., 1929, xxi, 567.
CAULK AND GRUBER: Nelson's Looseleaf Surgery, 1928, vi, 693.
(215) BUDGE: Untersuchungen über des Nervensystem, 1841, i, 159; Virchow's. Arch. f. Exp. Path., 1858, xv, 115; Zeitschr. f. rat. Med. v. Henle u. Pfeufer, 1864, iii, R., xxi, 1, 174; ibid., 1865, xxiii, 78; Compt. rend. 1864, i, 529; Pflüger's Arch., 1872, vi, 306.
(216) KUPRESSOW: Pflüger's Arch., 1872, v, 291.
(217) MACDONALD AND M'CREA: Quart. Journ. Exp. Physiol., 1930, xx, 379.
(218) KISSELEW: Zentralbl. f. d. Med. Wissenschr., 1868, xxii, 337.
(219) GSCHEIDLEN: Arch. f. Mikrosk. Anat., 1877, xiv, 321.
(220) MICHAILOW: Arch. f. Mikros. Anat., 1908, lxxi, 72; ibid., 1908, lxxii, 554.
(221) HOFBAUER: Journ. Urol., 1928, xx, 413.
(222) KRAUSE: Anat. des Kaninchens, Leipzig, 1884, ii, 355; Zeitschr. f. rat. Med., Henle u. Pfeufer, 1866, xxviii, 86.
(223) BOECKE: Zeitschr. f. Mikros. Anat. Forsch., 1925, ii, 391; Proc. Roy. Acad. and Sci., 1915, xvii, 32.
(224) PARKER AND HASWELL: Textbook of zoology, London, 1897, ii, 445–550.
(225) ROSSI: Journ. Comp. Neurol., 1922, xxxiv, 493.
(226) BODIN: Kubanski Wissenschaft Med., Westnik., 1924, iv, quoted by WOLHYNSKI: Zeitschr. f. Anat., 1930, xciii, 297.
(227) NAWROCKI AND SKABITSCHEWSKY: Pflügers Arch., 1891, xlviii, 335; ibid., 1891, xlix, 141.
(228) LANGLEY: Journ. Physiol., 1891, xii, xxiii; Schaefer's Textbook of physiology, 1900, ii, 679.
(229) LANGLEY AND ANDERSON: Journ. Physiol., 1893–94, xv, xi; ibid., 1894, xvi, 410; ibid., 1894, xvii, 177; ibid., 1895, xviii, 67; ibid., 1895–96, xix, pp. 71, 131 and 372; ibid., 1896, xx, 372.
(230) DENNING: Die Innervation der Harnblase, Springer, 1926, 98; Physiol. u. Klinik. Monog. a.d. geb. d. Neurol. u. Psychiat., Berlin, 1926, xlv, 1; Wien. Med. Wochenschr., 1926, lxxvi, 1140.
(231) SOKOWNIN: Pflüger's Arch., 1874, viii, 600. Reviewed by NAWROCKI: Hofmann Schwalbe Jahresb., 1877, vi, 2, 87.
(232) STEWART: Amer. Journ. Physiol., 1899, ii, 182; ibid., 1900, iii, 1; ibid., 1901, iv, 185.
(233) WOLHYNSKI: Zeitschr. f.d.g. Anat., (Abt. 1), 1930, xciii, 297.
(234) REHFISCH: Arch. f. Path. Anat. u. Physiol., 1897, cl, 111; ibid., 1900, clxi, 529.
(235) SCHABADASCH: Cited by MEDOWAR: Zeitschr. f. d. ges. Anat., Anat. Abt., 1928, lxxxvi, 784.
(236) PISEMSKY: Sur. l. innervation de la Matrice, Kupp, 1904.
(237) NIKOLSKY: Arch. f. Anat. u. Physiol., Physiol. Abt., 1879, 209.
(238) MEDOWAR: Zeitschr. f. d. ges. Anat., 1928, lxxxvi, 776.
(239) GIANUZZI: Journ. d. l. Physiol., 1863, vi, 22; Ricerche eseguite nel gabinetto di fisiologica della R. Universita di Siena. Anno. 1868–69; Rivista Scientifica, Anno I, Fasc, I, Siena 1869, i, 1.
(240) NUSSBAUM: Hoffmann Schwalbe's Jahresbericht, 1879, viii, 64; Denkschrift der Warz. Aertzl. gesellsch., 1880, lxxxvi, 222.
(241) BARRINGTON: Quart. Journ. Exp. Physiol., 1914, viii, 33.

(242) Dale: Journ. Physiol., 1906, xxxiv, 179.
(243) Sherrington: Brit. Med. Journ., 1891, 1016; Journ. Physiol., 1892, xiii, 621.
(244) Sollmann: Physiol. Reviews, 1923, ii, 479.
(245) Gaskell: Journ. Physiol., 1886, vii, 1; ibid., 1887, viii, iv.
(246) Eckhard: Beitr. z. Anat., 1863, iii, 123; ibid., 1869, iv, 69; ibid., 1876, vii, 67.
(247) Bernheim: Arch. f. Physiol., Suppl. 1892, 11.
(248) Steinach and Wiener: Pflüger's Arch., 1895, lx, 593.
(249) Horton-Smith: Journ. Physiol., 1897, xxi, 101.
(250) Dale: Journ. Physiol., 1901, xxvii, 350.
(251) Gaskell: Journ. Physiol., 1886, vii, 12, 13, 26, 29.
(252) Langley and Orbeli: Journ. Physiol., 1910, lxi, 474.
(253) Waters: Journ. Physiol., 1885, vi, 460.
(254) Oehl: Compt. rend., 1865, ii, 340; Schmidt's Jahrbuch., 1869, cxli, 274; Henle's, Meissner's and Grenacher's Bericht ü. d. Fortschritte des Anat. u. Physiol., in Jahre 1869. Leipzig u. Heidelburg 1871, 303.
(255) Palumordwinov: Neurologischeski Vest., 1916, xxii, xxiii, reviewed in Physiol., Abst., 1917–18, ii, 20.
(256) Kehrer: Zeitschr. f. rat. med. Henle u. Pfeufer, 1867, iii, R, xxix, xix, 144.
(257) Bert: Arch. d. Physiol., 1869, ii, 650.
(258) Remak: Schmidt's Jahrb., 1840, xxvii, 13.
(259) Darwin: Quart. Journ. Micros. Sci., 1874, xiv, 109.
(260) Stöhr: Zeitschr. f. d. ges. Anat., 1926, lxxviii, 555.
(261) Müller: Deutsch. Arch. f. klin. Med., 1918–19, cxxviii, 81; Sitzungsb. Physik. Med. ges. Würzburg, 1916–17, 59.
(262) Grünstein: Arch. f. Mikros. Anat., 1900, lv, 1.
(263) Wolff: Arch. f. Mikros. Anat., 1882, xx, 361.
(264) v. Lowdowsky: Arch. f. Anat. u. Physiol., 1872, 55.
(265) Serebrjakow: Zeitschr. f. Zellforsch. u. Mikr. Anat., 1929, ix, 425.
(266) Arnold: Stricker's Handbuch. f. Gewebelehre, 1871, i, 142.
(267) Lustig: Sitzungsb. d. Kais. Adak. d. Wiss. Wien, 1881, lxxxiii, 186.
(268) Obregia: Verhandl. des. 10 Inter. Med. Kongr. Berlin, 1890.
(269) Loewi: Sitzungsb. d. Kais. Acad. d. Wiss. Wien. 1875, lxxi, 355.
(270) Huber: Journ. Comp. Neurol., 1897, vii, 73.
(271) v. Csiky: Cited by Stöhr, Zeitschr. f. d. ges. Anat., 1926, lxxviii, 571.
(272) Agababow: v. Grafe's Arch. f. Ophthal. 1912, lxxxiii, 317.
(273) Courtade and Guyon: Arch. d. Physiol. 1896, viii, 622.
(274) Morgan and Dreschfeld: Brit. Med. Journ., 1881, i, 152.
(275) Schultz: Arch. f. Physiol., 1895, 542.
(276) Gruber: Journ. Pharm. Exp. Therap., 1926, xxx, 154.
(277) Sertoli: Arch. ital. d. biol., 1883, iii, 81.
(278) Botazzi: Memorie della K. Acad. d. Lincei, 1915, xi, 43; Arch. ital d. biol., 1916, lxv, 265.
(279) Straub: Pflüger's Arch., 1907, cxix, 127.
(280) Stewart: Amer. Journ. Physiol., 1905, x, 195.
(281) Mosso and Pellicani: Arch. ital. d. biol., 1882, i, 97, 291; Real Academia d. Linei, 1882, 220; Sulle funzioni della vesica 1882, 43.
(282) Beattle: Can. Med. Assoc. Journ., 1930, xxiii, 71.

(283) Fearnsides: Brain, 1917–18, xl, 149.
(284) Head and Riddock: Brain, 1917, xl, 188.
Riddock: Journ. Physiol., 1921, liv, cxxxiv.
(285) Ott: Journ. Physiol., 1879, ii, 44; Med. Bull. Philadelphia., 1894, xvi, 410.
(286) Spiegel and MacPherson: Pflüger's Arch., 1925, ccviii, 570.
(287) Masius: Bull. d. l. Acad. royal d. Sci. d. Belgique, 1867, xxiv, 312; ibid., 1868, xxv, 491.
(288) Goltz and Freusberg: Pflüger's Arch., 1874, viii, 460.
(289) Landois: Lehrbuch. d. Physiol. d. Menschen, 1891, vii, 550.
(290) Jackson: Surg., Gyn. and Obstet., 1917, xxv, 346.
(291) v. Zeissl: Ann. Mel. Org. Genito-urinaires, 1892, 828; Wien. Med. Wochenschr., 1901, 465, 1201; Wien. Klin. Wochenschr., 1901, xiv, 147; Pflüger's Arch., 1893, liii, 560; ibid., 1894, lv, 569; ibid., 1902, lxxxix, 605.
(292) Loven: Ber. ü. d. Verhandl. d. Königl. Sächs. gesell. d. Wissenschr. z. Leipzig, 1866, 8.
(293) François-Frank: Compt. rend. soc. d. biol., 1894, xlvi, 740; Arch. d. Physiol. norm. e. path. 1895, xxvii, 122.
(294) Clark: Journ. Anat. Physiol., 1883, xvii, 442.
(295) Frankl-Hochwart and Fröhlich: Neurol. Centralbl., 1904, xxiii, 646.
(296) Longet: Anat. e. Physiol. d. Système Nerveux, 1842, ii, 614.
(297) Hanč: Pflüger's Arch., 1898–99, lxxiii, 453.
(298) Guyon: Compt. rend. soc. de biol., 1900, 712.
(299) Heidenhain and Colberg: Arch. f. Anat., 1858, 437.
(300) Barger and Dale: Biochem. Journ., 1907, ii, 250.
(301) Streuli: Zeitschr. f. Biol., 1915, lxvi, 167.
(302) Chiari and Fröhlich: Arch. f. Exp. Path. u. Pharm., 1911, lxiv, 214.
(303) Langley: Cited by Cushny: Journ. Physiol., 1910, xli, 235.
(304) Henderson: Journ. Pharm. Exp. Therap., 1922, xix, 271.
(305) Young and Macht: Journ. Pharm. Exp. Therap., 1924, xxii, 329.
(306) Adler: Arch. f. Exp. Path. u. Pharm., 1918, lxxxiii, 248; Monatschr. f. Gynäk., 1912, xxxvi, 133.
(307) Fröhlich and Loewi: Arch. f. Exp. Path., u. Pharm., 1908, lix, 34.
(308) Brachet: Rech. Expérin. s. l. Syst. Nerv. gangl. 1830, p. 262, ibid., 1837, i, 262; ibid., 1839, ii, 290.
(309) Hall: Abhandlungen über des Nerven System. Aus dem Englischen von Kürschner, Marburg 1840, pp. 36, 39, 74.
(310) Spina: Cited by Retterer; Richet's dictionnaire 1897, v, 519.
(311) Akutsu: Pflüger's Arch., 1903, xcvi, 541.
(312) Bacq: Amer. Journ. Physiol. 1931, xcvi, 321.
(313) Morat: Arch. d. Physiol. norm. et path., 1890, ii, Series 5, 493.
(314) Ostroumoff: Pflüger's Arch., 1876, xii, 219.
(315) Anrep and Czybulski: St. Petersb. Med. Wochenschr., 1884, cited by Piotrowski: Pflüger's Arch., 1894, lv, 240.
(316) Piotrowski: Pflüger's Arch., 1894, lv, 240.
(317) Fletcher: Journ. Physiol., 1897–98, xxii, xxxvii.
(318) Fisher: Anat. Record, 1917, xiii, 69.
(319) De Zilwa: Journ. Physiol., 1901, xxvii, 200.
(320) Edmunds: Journ. Pharm. Exp. Therap., 1920, xv, 201.

(321) Brücke: Pflüger's Arch., 1910, cxxxiii, 313; ibid., 1910, cxxxvi, 502.
(322) Starling: Textbook of physiology, 1915, ii, 247.
(323) Waddell: Journ. Pharm. Exp. Therap., 1917, ix, 179.
(324) Marshall: The physiology of reproduction, 1910, 258.
(325) Barrington: Internat. Monatschr. f. anat. u. physiol., 1913, xxx, 1.
(326) Mislawsky and Bormann: Zentralb. f. Physiol., 1898, xii, 181.
(327) Macht: Journ. Urol., 1922, vii, 407.
(328) Griffiths: Journ. Anat. Physiol., 1890, xxiv, 35.
(329) Retzius: Internat. Monatschr. f. Anat. u. Physiol., 1890, vii, 323.
(330) Timofejew: Anat. Anz. 1894, ix, 342; ibid., 1896, xi, 44; Zeitschr. f.d.g. Anat. 1897, vii, 627.
(331) Takahashi: Pflüger's Arch., 1922, cxcii, 322; ibid., 1922, cxcvi, 237.
(332) Marconi: Compt. rend. soc. Biol., 1923, lxxxviii, 356.
(333) Lipschütz and Vass: Compt. rend. soc. biol., 1924, xc, 201.
(334) Ohmora: Zeitschr. f. Anat. u. Entwicklg. 1924, lxx, 347.
(335) Wlasow: Centralbl. f. Physiol., 1904, xviii, 776.
(336) Wolhynski: Zeitschr. f. d. ges. Anat., 1930, xciii, 297.
(337) Schäfer: Quain's Anatomy, 1902, 124.
(338) Sisson: The anatomy of domestic animals, 1914, 579.
(339) Waddell: Journ. Pharm. Exp. Therap., 1917, ix, 113, 179.
(340) Jordon and Ferguson: Textbook of histology, 1916, 495.
(341) Fränkel: Zeitschr. f. Morphol. u. Anthropol., 1903, v, 346.
(342) Starling: Schäfer's Textbook of physiology, 1900 ii, 347.
(343) Remy: Journ. d. l'anat. e. Physiol., 1886, xxii, 205.
(344) Segalas: Journ. d. Physiol. Exp. Pharm., 1842, iv, 293.
(345) Macht: Journ. Pharm. Exp. Therap., 1917, ix, 121.
(346) Waddell: Journ. Pharm. Exp. Therap., 1916, iii, 551.
(347) Sclavunos: Anat. Anz., 1894, ix, 42.
(348) Nagel: Arch. f. Physiol., 1905, Suppl., 458; Handbuch. d. Physiol. d. Mensch., 1906–07, ii, 74.
(349) Loeb: Dissertation Giessen, 1866, quoted by Nagel; Handb. d. Physiol. d. Mensch., 1906–07, ii, 74.
(350) Fick: Arch. f. Anat. u. Physiol., 1856, 473.
(351) Macht: Journ. Urol., 1917, i, 97.
(352) Macht and Cook: Journ. Pharm. Exp. Therap., 1931, xliii, 598.
(353) Gohara: Acta Scholae Medicinalis, 1919, iii, 329.
(354) Cushny: Journ. Physiol., 1906–7, xxxv, 1; ibid., 1910, xli, 233.
(355) Retzius: Internat. Monatschr. f. Anat., u. Physiol., 1890, vii, 333.
(356) Geller: Zentralb. f. Gynäkol., 1922, xlvi, 623.
(357) Sfameni: Monit. Zool. ital., 1901, xii, 5.
(358) Worthman: Arch. f. Mikroskop. Anat., 1906, lxviii, 122.
(359) Dahl: Zeitschr. f. Geburtsh. u. Gynäkol. 1916, lxxviii, 539; Die Lebensnerven, 1924.
(360) Mabuchi: Mitt. a.d. Med. Fak., Tokio, 1924, xxxi, 385.
(361) Kadanoff: Zeitschr. f. Zellforsch. u. Mikr. Anat., 1930, x, 427.
(362) Kenuira: Jap. Journ. Obst. Gynec., 1930, xiii, 90.
(363) Kraul: Arch. f. Gynäk., 1928, cxxxi, 600.
(364) Wallart: Arch. f. Gynäk., 1930, cxliii, 176.

(365) DèVos: Acad. Roy. d. Med. d. Belgique, 1894, viii, 552.
(366) Riese: Anat. Anzeiger, 1891, vi, 401.
(367) v. Herff: Zeitschr. f. Geburtsh. u. Gynäk., 1892, xxiv, 289.
(368) Mandl: Centralb. f. Gynäk., 1894, xxviii, 677.
(369) v. Gawronsky: Centralbl. f. Gynäk., 1894, ii, 230.
(370) Crossen: Diseases of women, 1919, iv, 919.
(371) Retzius: Biol. Untersuchen Neu. Folge, 1893, v, 31.
(372) Lhermitte and Dupont: Gynéc. e. Obst., 1927, xv, 161; Journ. Amer. Med. Assoc., 1926, lxxxvii, 65.
(373) Langley and Orbeli: Journ. Physiol., 1910–11, xli, 450.
(374) Harting: Zeitschr. f. Zellforsch. u. Mikros. Anat., 1929, ix, 544.
(375) Kok: Arch. f. Gynäkol., 1925, cxxvii, 384; ibid., 1927, cxxx, 173.
(376) v. Ebner: Kölliker's Handb. d. Gewebelehre, 1902, iii, 1.
(377) Köstlin: Fortschr. Med., 1894, xii, 411.
(378) Jacquis: Bibliogr. Anat., 1894.
(379) Kuhn: Inaug. Diss. Berlin, 1906.
(380) Roith: Arch. f. Gynäk., 1907, lxxxi, 495; Monatschr. f. Geburtsh. u. Gynäk., 1907, xxv, 79.
(381) Gawronsky: Arch. f. Gynäk., 1894, xlvii, 271.
(382) Coryllos: Rev. franc. de Gynécol., 1913, xxvii, 257.
(383) Ries: Zeitschr. f. Geburtsh. u. Gynäk., 1908, lxii, 100.
(384) Gohara: Kink. Fuzinkwa gakkwai Kwaiho, 1919, vii, 328; cited by Gohara, loc. cit.
(385) Rainey: Philosoph. Trans., 1848.
(386) Junkmann and Strass: Klin. Wochenschr., 1925, iv, 1119.
(387) Jung: Monatschr. f. geburtsh. u. Gynäk., 1905, xxi, 1.
(388) Jastreboff: Arch. f. Anat. u. Physiol., Physiol., Abt., 1884, 90.
(389) Keiffer: Bull. Soc. d. Obstet., 1908, xi, 50.
(390) Kehrer, F. A.: Inaug. Diss. Giessen, 1864, quoted by Jastreboff.
(391) Kehrer, E.: Arch. f. Gynäk., 1906–07, lxxxi, 160.
(392) v. Swiecicki: Zeitschr. f. Geburtsh. u. Gynäk., 1884, x, 301.
(393) Bechterew and Miszlawsky: Medic. Obosrinje 18911 Russ.
(394) Fellner: Centralbl. f. d. Med. Wissensch., 1887, xxv, 259.
(395) Gunn and Franklin: Proc. Roy. Soc., 1922, B, xciv, 197.
(396) Falk: Zeitschr. f. Geburts. u. Gynäk., 1913, lxxiv, 881; Journ. Obstet. Dis. Women (Russian) 1914, xxix, 73.
(397) Gunn and Davies: Journ. Physiol., 1920, liv, lxxxvi.
(398) Van Dyke: Journ. Pharm. Exp. Therap., 1926, xxvii, 305.
(399) Waddell: Journ. Pharm. Exp. Therap., 1917, ix, 411.
(400) Tyler-Smith: The Lancet, 1848, 268.
(401) Körner: Studien d. physiol. Institut. zu Breslau, 1865; De Nervis uteri. Diss. inaug. Vratislav, 1864.
(402) Röhrig: Virchow's Arch., 1879, lxxvi, 1.
(403) Bayer: Freund's Gynäk. Klin., 1885, 369.
(404) Kreitzer: Petersburg med. Zeitschr., 1871, 113.
(405) Veit: Müller's Handbuch d. Geburtshülfe, 1888, i, 122.
(406) Ribemont-Dessaignes: Précis, d'obstétrique, Paris, 1894, 30.

(407) Bradly: Topographical anatomy of the dog, 1907.
Scissin: The anatomy of domestic animals, 1917.
Fleming: Veterinary obstetrics, 1912.
Williams: Veterinary obstetrics, 1909.
(408) Hoogkamer: Arch. f. Gynäkol., 1913, xcix, 231.
(409) Elischer: Centralb. f. d. Med. Wissenschr., 1876, xiv, 884.
(410) Elischer: Centralb. f. d. Med. Wissenschr., 1876, xiv, 493; Arch. f. Gynäk., 1876, ix, 10.
(411) Acconci: Giorn. della Real Acad. d. Med. d. Torino, 1891, vii, 497, 708.
(412) Razumowski: Dissertation Petersburg, 1881.
(413) Gemmell: Journ. Obst. Gynec. Brit. Emp. 1926, xxxiii, 259.
(414) Naiditsch: Arch. f. Gynäk., 1930, cxxxix, 283.
(415) Labhardt: Arch. f. Gynäk., 1906, lxxx, 135.
(416) Bruckner and Mezincescu: Compt. rend. soc. d. boil., 1903, lv, 323, 324.
(417) Oudendal: Nederl. Maandschr. Gennsk. Leiden, 1922, 11.
(418) Harvey: Cited by Haller: Elementa Physiologiae Corp. hurn. 1657, T. vii, l, xxviii, 60. Cited by Frommel: Zeitschr. f. Geb. u. Gyn., 1882, viii, 205.
(419) Venette: Cited by Haller: Elementa Physiol. Corp. hum. 1657, viii, l, 28, 60; cited by Frommel: Zeitschr. f. Geb. u. Gynäk., 1882, viii, 20.
(420) Graaf: Cited by Haller: Elementa Physiologiae Corp. hum, 1657, T. viii, l, xxviii, 10, 60; ibid., T. iv, 446; ibid., T. viii, xxix, 420.
(421) Kilian: Zeitschr. f. rationelle Med. Henle u. Pfeufer, 1851, x, 45; ibid., 1850, ix, 1; ibid., 1852, ii, 1.
(422) Franz: Zeitschr. f. Geburtsh., 1904, liii, 361.
(423) Snow-Beck: Medical Times, 1851; Philosoph. Trans., 1846, 213.
(424) Barlow: The Lancet, 1847, i, 664.
(425) Heddaeus: Die contraction der gebärmutter in physiologischer Bezichung, etc., Diss. inaug. Würzburg, 1851.
(426) Bertling: Nonnulla experimenta, etc., Diss. Inaug. Marburg, 1853.
(427) Budge: Untersuchungen über die Nervensystem, Frankfurt, 1841, i, 174.
(428) Spiegelberg: Monatschr. f. Geburtsh., 1864, 24; Zeitschr. f. rat. med. Henle u. Pfeufer, 1858, ii, 1.
(429) Barbour and Copenhaver: Proc. Soc. Exper. Biol. Med., 1916, xiii, 159.
(430) Obernier: De Nervis uteri, Diss. inaug. Bonn, 1862.
(431) Obernier: Exp. Iterus ü. d. Nerven des Uterus, Diss. Bonn. 1865.
(432) Goltz and Freusberg: Pflüger's Arch., 1874, ix, 552.
(433) Friedenrich and Nasse: Untersuch. z. Physiol. u. Path., 1835, ii, 268.
(434) v. Basch and Hofmann: Wiener Med. Jahrb. 1877, 465.
(435) Kurdinowsky: Physiol. u. Pharm. Exp. a. isol. Uterus. Diss. Petersburg, 1903; Arch. f. Gynäk. 1904, lxxiii, 425; ibid., 1907, lxxxi, 340; ibid., 1906, lxxviii, 34, 539.
(436) Caliburcès: Recherches Exp. etc. Compt. rend. hebdom. d. acad. d. sciences, 1857, 45.
(437) Rein: Pflüger's Arch., 1880, xxiii, 68.
(438) Haller: Elementa Physiol. Lausannae, 1762, iv, 266.
(439) Frommel: Zeitschr. f. Geburtsh. u. Gynäk., 1882, viii, 205.
(440) Kronecker: Arch. f. Physiol., 1883, 259.

(441) Chidichines: Arch. ital. d. biol., 1904, xlii, 323.
(442) Eustachius: Tabulae Anatomicae Amstelaedami, 1722.
(443) Reil: Arch. f. Physiol., 1807, vii, 189.
(444) Scanzoni: Prager Vierteljahro, 1849, xxiv, 4; Lehrb. b. d. Geburtsh. 1867, i, 218.
(445) Ellinger: Kristische u. Exp. Stud. z. physiol. d. uterus, Berlin, 1888.
(446) Nowland: Journ. Coll. Surg. Australia, 1929, ii, 173.
(447) Whitehouse and Featherstone: Journ. Obst. and Gynaec. Brit. Emp., 1923, xxx, 565.
(448) Cyon: Pflüger's Arch., 1874, viii, 349.
(449) Brown: Sympathetic nervous system in disease.
(450) Dale and Laidlaw: Journ. Physiol., 1912, xlv, 1.
(451) Sugimoto: Arch. f. Exp. Path. u. Pharm., 1913, lxxiv, 27.
(452) Markee: Amer. Journ. Physiol., 1932, c, 32; Amer. Journ. Obstet. and Gynec. 1929, xvii, 205.
(453) Meyer and Gottlieb: Pharmacology, clinical and pharmacological. Transl. by Halsey, 1914, 222.
(454) Dyroff: Arch. f. Gynäk., 1929, cxxxviii, 362.
(455) Reismann: Einige Untersuch. ü. Nervöse un. andere Erreger von uterus kontraktionen, Diss. Kiew. 1861; Arch. f. Gynäk. 1871, ii, 97.
(456) Lundberg: Compt. rend. soc. d. biol., 1925, xcii, 647.
(457) Ishikawa: Arb. a. d. Neurol. Inst. a. d. Wien. Univ. 1928, xxx, 327.
(458) Aburel: Compt. rend. soc. d. biol., 1930, ciii, 902.
(459) Biagi: Biochem. Centralbl., 1905, iv, 762.
(460) Okamoto: Act. Schol. Med. Kyoto, 1918, ii, 307.
(461) Nikolaeff: Arch. f. Exp. Path. u. Pharm., 1931, clx, 569.
(462) Cow: Journ. Physiol., 1919, lii, 301; ibid., 1914, xlviii, 443.
(463) M'Swiney and Brown: Journ. Physiol., 1926, lxii, 52.
(464) Gunn and Gunn: Journ. Pharm. Exp. Therap., 1913–14, v, 527.
(465) Rübsamen and Danziger: Zeitschr. f. Geburtsh. u. Gynäk. 1911, lxix, 656.
(466) Rübsamen and Klingermann: Zeitschr. Geburtsh. u. Gynäk., 1912, lxxii, 272.
(467) Lieb: Amer. Journ. Obst., 1915, lxxi, 217.
(468) Gunn: Proc. Royal Soc., 1914, B, lxxxvii, 551.
(469) Gruber: Endocrinol., 1925, ix, 407.
(470) Rucker: Amer. Journ. Obst. and Gynec., 1927, xiv, 609.
(471) Ivy, Hartman and Koff: Amer. Journ. Obst. and Gynec., 1931, xxii, 388.
(472) Rudolph and Ivy: Amer. Journ. Obst. and Gynec., 1930, xix, 317.
(473) Knaus and Clark: Journ. Pharm. Exp. Therap., 1926, xxvi, 347.
(474) Falta and Flemming: Münch. Med. Wochenschr., 1911, lviii, 2649.
(475) Hoskins: Journ. Amer. Med. Assoc., 1927, lxxxviii, 2011.
(476) Braun: Arch. f. Exp. Path. u. Pharm., 1925, cviii, 96.
(477) Tate and Clark: Arch. inter. d. Pharm. e. d. Therap. 1922, xxvi, 103.
(478) Gaddum: Journ. Physiol., 1926–27, lxi, 141.
(479) Fröhlich and Pick: Arch. f. Exp. Path. u. Pharm. 1913, lxxi, 23.
(480) Sandström: Compt. rend. soc. d. biol., 1927, xcvii, 1646.
(481) Knaus: Arch. f. Exp. Path. u. Pharm., 1927, cxxiv, 152.
(482) Fardon: Biochem. Journ., 1908, iii, 405.

(483) Quadiarullo: Arch. d. Obstet. genèc., Naples, 1918, v, 1; Physiol. Abstracts, 1916–17, i, 330; ibid., 1918–19, iii, 454.
(484) Sellheim: Negel Handbuch. d. Physiol. d. Menschr., 1907, ii, 110.
(485) Erdheim and Stumme: Berl. Klin. Wochenschr., 1908, xlv, 1031; Zeigler's Beitr. 1909, xlvi, 1.
(486) Marek: Centralbl. f. Gynäk., 1911, xxxv, 1612.
(487) Bell: Sex complex. London, 1916, 55.
(488) v. Frankel-Hochwart and Fröhlich: Arch. f. Exp. Path. u. Pharm., 1910, lxiii, 347; Wien. Klin. Wochenschr., 1909, xxii, 982.
(489) Van Dyke and Gustavson: Journ. Pharm. Exp. Therap., 1929, xxxvii, 379.
(490) Merdler: Zentralb. Gynäk., 1923, xlvii, 1824.
(491) Ransom: Journ. Pharm. Exp. Therap., 1920, xv, 181.
(492) Zodenk: Biochem. Zeitschr., 1922, cxxxii, 262.
(493) Turolt: Arch. v. Gynäk., 1922, cxv, 600.
(494) Kehrer: Arch. f. Gynäk., 1920, cxii, 487.
(495) Bell: Brit. Med. Journ., 1913, vi, 707.
(496) Bell and Hicks: Brit. Med. Journ., 1909, i, 777.
(497) Hyernaux: Centralbl. f. Gynäk., 1877–78, i, 613; Bull d. l'acad. Royale d. Med. d. Bruxelles, 1878, xii, 701.
Kleinwächter: Arch. f. Gynäk., 1878, xiii, 280.
v. Massmann: Centralb. f. Gynäk., 1878, ii, 193.
v. Felsenreich: Wien. Med. Wochenschr., 1878, xxviii, 23.
Höhne: Schmidt's Jahrb., 1878, 273.
Schnabel: Über die Einleitung der kunstlichen Freihgeburt durch Pilocarpine., Diss. Tübigen, 1879.
Brenneke: Berl. klin. Wochenschr., 1880, xvii, 122.
(498) Cushny: Brit. Med. Journ., 1906, ii, 1460.
(499) Stewart and Rogoff: Journ. Pharm. Exp. Therap., 1920–21, xvi, 71; ibid., 1916, viii, 205.
(500) Tscheboksaroff: Pflüger's Arch., 1910–11, cxxxvii, 59.
(501) Elliott: Journ. Physiol., 1912, xliv, 374; ibid., 1911, xliii, p. xxxii.
(502) Ehrmann: Arch. f. Exp. Path. u. Pharm., 1906, lv, 44.
(503) Kuyer and Wijsenbeek: Pflüger's Arch., 1913, cliv, 16.
(504) Seel: Arch. f. Exp. Path. u. Pharm., 1926, cxiv, 362.
(505) Halste: Arch. f. Exp. Path. u. Pharm., 1924, ci, 36.
(506) Hasama: Arch. f. Exp. Path. u. Pharm., 1925, cvi, 223; ibid., 1930, cliii, 140.
(507) Bradford: Journ. Physiol., 1887, viii, 86.
(508) Valanti: Monit. Zool. itial., 1926, xxxvii, 47.
(509) Abelin: Zeitschr. f. Biol., 1919, lxix, 391.
(510) Dastre and Morat: Compt. rend. Acad. d. Sci., 1880, xci, 393.
Heidenhain: Pflüger's Arch., 1878, xvi, 1.
(511) Latarjet and Bertrand: Lyon Chir., 1923, xx, 452.
(512) Langley and Dickinson: Proc. Royal Soc., 1890, xlvi, 423.
(513) Brown-Séquard: Arch. d. Physiol., 1890, ii, 411.
(514) Rauther: Jena Zeitschr Naturwiss., 1913, xxxvii, 377.
(515) Gerhardt: Monograph einheimischer Tiere, Leipzig, 1909.
(516) Lee: Philosoph. Trans., 1841, i, 272.
(517) Hinsey and Markee: Proc. Soc. Exp. Biol. Med., 1932, xxx, 136.
(518) Henderson: Report, Meeting Amer. Soc. Pharm. Exp. Therap., 1933.

MEDICAL LIBRARY

OCT 20 1933

PHYSIOLOGICAL REVIEWS

EDITED FOR

THE AMERICAN PHYSIOLOGICAL SOCIETY

CONTENTS

PAGE

PHYSICAL FACTORS IN BODY TEMPERATURE MAINTENANCE AND HEAT ELIMINATION. *Thomas Deighton* 427

RADIATION-GENETICS. *Frank Blair Hanson* 466

THE AUTONOMIC INNERVATION OF THE GENITO-URINARY SYSTEM. *Charles M. Gruber* .. 497

VOL. XIII, No. 4

Issued October, 1933

BALTIMORE, U. S. A.

Entered as second-class matter, January 15, 1921, at the Post Office at Baltimore, Md., under the act of March 8, 1879

Made in United States of America

PHYSIOLOGICAL REVIEWS

PHYSIOLOGICAL REVIEWS is owned and controlled by the American Physiological Society. It is edited by a board appointed by the society consisting of:

A. J. CARLSON, Chicago
LEO LOEB, St. Louis
J. J. R. MACLEOD, Aberdeen, Scotland
H. S. GASSER, New York
W. R. BLOOR, Rochester
C. D. LEAKE, San Francisco
D. R. HOOKER, Managing Editor, Baltimore

The Editorial Board selects the subjects and authors of all articles which are published, the aim being to provide concise but comprehensive reviews of the recent literature and present status of various subjects in Physiology, using this term in a broad sense to include Bio-chemistry, Bio-physics, Experimental Pharmacology and Experimental Pathology. Each volume will consist of from sixteen to twenty articles, making a total of about five hundred pages per volume. The numbers will be issued quarterly in January, April, July and October of each year.

Subscriptions will be received in advance only. The subscription price is:

$6.00 per volume in the United States, net postpaid
$6.25 per volume in Canada, net postpaid
$6.50 per volume elsewhere, net postpaid
$2.50 per single number, net postpaid

Subscriptions should be sent to

D. R. HOOKER, *Managing Editor*
19 W. CHASE STREET, BALTIMORE, MD.

NUTRITION ABSTRACTS & REVIEWS

Issued Under the Direction of the Imperial Agricultural Bureaux Council, the Medical Research Council and the Reid Library

EDITORS

JOHN BOYD ORR, Imperial Bureau of Animal Nutrition, Aberdeen.
J. J. R. MACLEOD, University of Aberdeen.
HARRIETTE CHICK, Lister Institute, London.

The Editors, in co-operation with Corresponding Editors abroad scrutinize upwards of 400 periodicals and, with the assistance of a competent panel of abstractors issue abstracts of papers published throughout the World dealing with the subject of nutrition in all its aspects.

The abstracts are conveniently arranged in three main sections: (1) Chemistry and Physiology of Nutrition, (2) Human Diet in Relation to Health and Disease, and (3) Feeding of Domestic Animals. These sections are designed to meet the requirements respectively of the research worker, the medical practitioner and dietitian and the practical farmer.

Four numbers appearing in July, October, January and April of each year constitute a Volume, the subscription price for which is 21/–.

The current volume is Volume III.

Subscriptions may be paid through any bookseller or direct to the Secretary, Committee of Management "Nutrition Abstracts & Reviews", Reid Library, Rowett Institute, Aberdeen, Scotland.

PHYSIOLOGICAL REVIEWS

Contents of Volume XI, 1931

An Index to Volumes I to X inclusive was published in the January issue

E. U. Still: Secretin

R. H. Jaffe: The Reticulo-Endothelial System in Immunity

T. H. Milroy: The Present Status of the Chemistry of Skeletal Muscular Contraction

O. H. Horrall: The Toxicity of Bile

C. F. Cori: Mammalian Carbohydrate Metabolism

M. Nordmann: Local Reactions in Sensitized Animals

B. A. McSwiney: Innervation of the Stomach

J. Argyll Campbell: The Tension of Gases in Tissue

E. Waldschmidt-Leitz: The Mode of Action and Differentiation of Proteolytic Enzymes

C. H. Best and E. W. McHenry: Histamine

Arthur L. Tatum and M. H. Seevers: Drug Addiction

Oscar Riddle: Factors in the Development of Sex and Secondary Sex Characteristics

Contents of Volume XII, 1932

A. D. Hirschfelder and R. N. Bieter: Local Anesthetics: Relation of Constitution to Action

D. P. Barr: Pathological Calcification

A. H. Baetjer: Effect of Muscular Fatigue upon Resistance

R. J. Anderson: Chemistry of Bacteria, Particularly the Fatty Acids of the Tubercle Bacillus

T. M. Rivers: The Constitution of Filterable Viruses

H. L. Blum: Photodynamic Action

B. Lucké and M. McCutcheon: The Living Cell as an Osmotic System and Its Permeability to Water

W. F. Petersen: Constitution and Disease

R. W. Gerard: Nerve Metabolism

F. R. Sabin: Cellular Reactions to Fractions Isolated from Tubercle Bacilli

D. Andersen: The Relationship Between the Thymus and Reproduction

A. V. Hill: The Revolution in Muscle Physiology

D. L. Thomson and J. B. Collip: The Functions of the Parathyroid Gland

H. D. Kay: Phosphatase in Diseases and Growth of Bone

Contents of Volume XIII, 1933

K. S. Lashley: Integrative Functions of the Cerebral Cortex

H. E. Roaf: Colour Vision

L. H. Weed: Positional Adjustments of the Pressure of the Cerebrospinal Fluid

A. H Steinhaus: Chronic Effects of Exercise

I. de Burgh Daly: Reactions of the Pulmonary and Bronchial Blood Vessels

G. F. Marrian: Recent Advances in the Chemistry and Biological Assay of Oestrin

W. A. Jacobs: The Chemistry of the Cardiac Glucosides

A. Claude and J. B. Murphy: Transmissible Tumors of the Fowl

F. J. McClure: A Review of Fluorine and Its Physiological Effects

H. E. Jordan: The Structural Changes in Striped Muscle during Contraction

J. G. Dusser de Barenne: The Mode and Site of Action of Strychnine in the Nervous System

E. F. Adolph: The Metabolism and Distribution of Water in Body and Tissues

C. E. Brunton: The Acid Output of the Kidney and the So-called Alkaline Tide

E. G. Wever: The Physiology of Hearing: The Nature of Response in the Cochlea

T. Deighton: Physical Factors in Body Temperature Maintenance and Heat Elimination

T. B. Hanson: Radiation-Genetics

C. M. Gruber: The Autonomic Innervation of the Genito-Urinary System

PHYSIOLOGICAL REVIEWS

Tentative Contents of Volume XIV, 1934

E. K. MARSHALL: The Aglomerular Kidney
C. L. A. SCHMIDT AND D. M. GREENBERG: Transport of Alkaline Earth Elements in the Animal Body
G. W. BARTELMEZ: Menstruation
L. B. FLEXNER: The Chemistry and Nature of the Cerebrospinal Fluid
H. JENSEN AND E. A. EVANS, JR.: The Chemistry of Insulin
R. G. SINCLAIR: Physiology of the Phospholipids
S. W. CLAUSEN: Diet and Infection
W. S. DUKE-ELDER: Application of Physiological Chemistry in the Study of Intraocular Pressure.
J. M. PETERSON: Metabolism of the Eviscerate Preparation
E. M. LANDIS: Capillary Pressure and Capillary Permeability
L. B. AREY: Mechanism of Wound Healing
J. C. HINSEY: The Innervation of Skeletal Muscle
G. A. ALLES: The Physiological Significance of Choline Derivatives
W. J. MEEK: Reflexes from the Vascular System
R. G. GARRY: The Movements of the Large Intestine
F. C. KOCH: The Male Sex Hormone
C. P. RICHTER: The Psycho-Galvanic Reflex
E. S. G. BARRON: Cellular Respiration
R. K. BURNS: Experimental Transformations of Sex in Vertebrates
G. W. CORNER: Hormonic Control of Mammary Secretion
J. J. MORTON AND W. J. M. SCOTT: Sympathetic Vasoconstriction in Normal and Pathological Arteries of the Extremities
C. E. BILLS: Physiology of Sterols and the Influence of Light on Sterols
A. C. IVY: Physiology of the Gall Bladder
S. MUDD, B. LUCKE AND M. MCCUTCHEON: Experimental Studies on Phagocytosis
A. HURTADO: Adaptation to High Altitude
H. S. LIDDELL: Recent Contributions to the Physiology of the Conditioned Reflex

Physiological Research Periodicals, complete files, back volumes and odd copies for sale. We purchase Medical and Scientific Magazines.

B. LOGIN & SON
29 EAST 21ST STREET, NEW YORK CITY